(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/016882 A1

(51) International Patent Classification[7]: G01N 21/59

(21) International Application Number: PCT/US02/22899

(22) International Filing Date: 18 July 2002 (18.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/312,165 14 August 2001 (14.08.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/016882 A1

(54) Title: MEASURING A SUBSTANCE IN A BIOLOGICAL SAMPLE

(57) Abstract: A method for measuring the amount of an organic substance in a biological sample with infrared electromagnetic radiation.

MEASURING A SUBSTANCE IN A BIOLOGICAL SAMPLE

TECHNICAL FIELD OF THE INVENTION

The present invention generally relates to a method of measuring an amount of an organic substance contained within a sample. The present invention particularly relates to a method of measuring the amount of an organic substance contained within a biological sample utilizing a limited number of selected infrared wavelength bands.

BACKGROUND OF THE INVENTION

An estimated 16 million Americans (approximately 7% of the total population in the United States) have diabetes, a disease which can cause severe damage to the heart, kidneys, eyes, and nerves. Diabetics need to monitor their blood glucose levels frequently, often as much as six times a day, to maintain a proper level of insulin in their blood. Intense testing and treatment of diabetes can reduce the complications, including blindness, kidney failure and heart attack, by as much as 70%.

Methods of measuring glucose are broadly divided into two categories: i.e., those based on chemical methods and those based on optical methods. Chemical methods of measuring blood glucose (e.g., an enzyme-based method) typically require the physical contact of a biological fluid with a sensing element utilized in the chemical method. One example of an apparatus which uses a chemical method is a blood glucose meter designed to measure the level of glucose in a sample of a patient's blood. In particular, a small amount of a suitable reagent is printed or otherwise deposited onto an elongate plastic strip which can be inserted into the blood glucose meter after contacting a blood sample from the patient. The meter includes a reflectometry based measuring system which detects a change in the color of the printed reagent due to a reaction between the active reagent and glucose present in the blood sample.

It should be appreciated that the accuracy of the meter is important where a patient determines an insulin treatment regime based upon blood glucose measurements obtained from a blood glucose meter. This requires very precise calibration of the meter. Initial calibration of the meter is normally carried out during

and immediately following manufacturing, with certain calibration data being stored in permanent memory of the meter. However, calibration of the meter at this stage cannot easily account for changes and variations in the properties of the consumable reagents themselves, variations which might arise due to slight changes in the manufacturing process of the reagent and the test strip, environmental factors such a temperature and humidity, and changes in the property of the reagent over time. Accordingly, measuring glucose levels with chemical methods may lead to inaccuracies.

Additional drawbacks to chemical methods include the manner in which they typically obtain the biological fluid. In particular, obtaining a blood sample typically involves pricking a finger of the diabetic patient. The aforementioned pricking is invasive, painful, and has a risk of infection. The self-pricking also requires a conscious and mindful patient. As such, chemical methods which involve pricking are inconvenient, and they often prevent the diabetic patient from performing the needed frequent testing. Moreover, because chemical methods typically include components which require periodic replacement, utilizing these methods can be costly.

With respect to optical methods, they typically relay upon absorption, scattering, and fluorescence to determine the content of glucose in a biological sample. These methods do not require physical contact with the sensing element, have relatively fast response times, and may require fewer calibration measurements. However, these methods suffer from the draw back that the equipment utilized to gather the optical data tends to be large, complex, and cumbersome and thus does not lend itself to being portable and easily used by a patient.

Therefore, in light of the above discussion, it is apparent that what is needed is a method of measuring an amount of an organic substance in a biological sample which address one of the above discussed drawbacks.

SUMMARY OF THE DISCLOSURE

In one illustrative embodiment, there is provided a method of measuring an amount of an organic substance contained within a biological sample. The organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of the wavelength regions substantially correspond

to an absorption band of the absorption spectrum. The method includes (a) detecting the intensity of a number of selected wavelength bands of infrared electromagnetic radiation influenced by the organic substance contained within the biological sample with a detection system, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions, and (ii) the number of the selected wavelength bands is equal to n-1 or less, (b) generating an electrical signal in response to detecting the intensity of the number of the selected wavelength bands, (c) receiving the electrical signal with a signal processor configured to process the electrical signal with a quantification algorithm, and (d) processing the electrical signal with the quantification algorithm so as to provide a measurement of the amount of the organic substance contained within the biological sample.

In another illustrative embodiment a method of measuring an amount of glucose in a biological fluid is provided. The glucose has an infrared absorption spectrum which includes a set (n) of infrared wavelength regions, wherein each of the infrared wavelength regions substantially correspond to an infrared absorption band of the infrared absorption spectrum. The method includes (a) detecting the transmittance of a number of selected wavelength bands of infrared electromagnetic radiation absorbed by the glucose contained within the biological fluid with a detection system, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions, and (ii) the number of the selected wavelength bands is equal to n-1 or less, (b) generating an electrical signal in response to detecting the transmittance of the infrared electromagnetic radiation, (c) receiving the electrical signal with a signal processor configured to process the electrical signal with a quantification algorithm, and (d) processing the electrical signal with the quantification algorithm so as to provide a measurement of the amount of the glucose contained within the biological fluid.

In yet another illustrative embodiment a method of measuring a concentration of an organic substance contained within a biological fluid is provided. The organic substance has an infrared absorption spectrum which includes a set (n) of infrared wavelength regions, wherein each of the infrared wavelength regions substantially correspond to an infrared absorption band of the infrared absorption spectrum. The method includes (a) detecting the transmittance of a number of selected wavelength bands of infrared electromagnetic radiation absorbed by the

organic substance contained within the biological fluid with a detection system, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions, and (ii) the number of the selected wavelength bands is equal to n-1 or less, (b) generating an electrical signal in response to detecting the transmittance of the selected infrared electromagnetic radiation wavelength bands, (c) receiving the electrical signal with a signal processor configured to process the electrical signal with a mathematical model, and (d) processing the electrical signal with the mathematical model so as to provide a measurement of the concentration of the organic substance contained within the biological fluid.

In yet another illustrative embodiment there is provided a method of measuring an amount of an organic substance contained within a biological sample. The organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of the wavelength regions substantially correspond to an absorption band of the absorption spectrum. The method includes (a) illuminating the biological sample with infrared electromagnetic radiation, wherein the infrared electromagnetic radiation includes (i) one or more wavelength bands of the infrared electromagnetic radiation which are absorbed by the organic substance contained within the biological sample, and (ii) one or more reference wavelength bands which are not substantially absorbed by the organic substance contained within the biological sample, (b) selecting a number the wavelength bands of the infrared electromagnetic radiation, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions and (ii) the number of the selected wavelength bands is a subset of (n), (c) selecting a number of reference wavelength bands, (d) detecting the intensity of only (i) the subset of the selected wavelength bands absorbed by the organic substance contained within the biological sample with a detection system, and (ii) the number of reference wavelength bands, (e) generating one or more electrical signals in response to detecting the intensity of only (i) the subset of the selected wavelength bands (ii) the number of reference wavelength bands, (f) receiving the one or more electrical signals with a signal processor configured to process the electrical signals with a quantification algorithm, and (g) processing the one or more electrical signals with the quantification algorithm so as to provide a measurement of the amount of the organic substance contained within the biological sample.

In still another illustrative embodiment a method of measuring an amount of an organic substance contained within a biological sample is provided. The organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of the wavelength regions substantially correspond to an absorption band of the absorption spectrum. The method includes (a) illuminating the biological sample with infrared electromagnetic radiation, (b) detecting the intensity of the infrared electromagnetic radiation that is absorbed by the organic substance contained within the biological sample, wherein (i) the intensity detection is restricted to a number of selected wavelength bands of infrared electromagnetic radiation, (ii) each of the selected wavelength bands substantially corresponds to one of the wavelength regions, and (iii) the number of the selected wavelength bands is a subset of (n), (c) generating an electrical signal in response to detecting the intensity of the subset of the selected wavelength bands, (d) receiving the electrical signal with a signal processor configured to process the electrical signal with a quantification algorithm, and (e) processing the electrical signal with the quantification algorithm so as to provide a measurement of the amount of the organic substance contained within the biological sample.

In still another illustrative embodiment a method of measuring an amount of an organic substance contained within a sample is provided. The organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of the wavelength regions substantially correspond to an absorption band of the absorption spectrum. The method includes (a) illuminating the sample with infrared electromagnetic radiation, wherein the infrared electromagnetic radiation includes (i) one or more wavelength bands of the infrared electromagnetic radiation which are absorbed by the organic substance contained within the sample (ii) one or more reference wavelength bands which are substantially not absorbed by the organic substance contained within the sample, (b) selecting a number the wavelength bands of the infrared electromagnetic radiation, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions and (ii) the number of the selected wavelength bands is a subset of (n), (c) selecting a number of reference wavelength bands, and (d) detecting with a detection system the intensity of only (i) the subset of the selected wavelength bands absorbed by the

organic substance contained within the sample and (ii) the number of reference wavelength bands.

In yet another illustrative embodiment a method of measuring an amount of an organic substance contained within a biological sample is provided. The organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of the wavelength regions substantially correspond to an absorption band of the absorption spectrum. The method includes (a) illuminating the biological sample with infrared electromagnetic radiation, wherein the infrared electromagnetic radiation includes (i) one or more wavelength bands of the infrared electromagnetic radiation which are absorbed by the organic substance contained within the biological sample and (ii) one or more reference wavelength bands which are substantially not absorbed by the organic substance contained within the biological sample, (b) selecting a number the wavelength bands of the infrared electromagnetic radiation, wherein (i) each of the selected wavelength bands substantially corresponds to one of the wavelength regions and (ii) the number of the selected wavelength bands is a subset of (n), (c) selecting a number of reference wavelength bands, (d) detecting with a detection system the intensity of the infrared electromagnetic radiation, and (e) processing with a mathematical model spectral data only from (i) the subset of the selected wavelength bands absorbed by the organic substance contained within the biological sample and (ii) the number of reference wavelength bands.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an ATR spectra of distilled water, LRS, glucose dissolved in distilled water, and glucose dissolved in LRS;

FIG. 2 is a schematic representation of a sensor;

FIG. 3 is another schematic representation of a sensor;

FIG. 4 is still another schematic representation of a sensor;

FIG. 5A is yet another schematic representation of a sensor;

FIG. 5B is still another schematic representation of a sensor;

FIG. 5C is yet another schematic representation of a sensor;

FIG. 6A is a graph showing calibration results;

FIG. 6B is a graph showing cross validation results;

FIG. 7A is a graph showing calibration results;

FIG. 7B is a graph showing cross validation results;

FIG. 8A is a graph showing pure quadratic calibration results;

FIG. 8B is a graph showing pure quadratic delete–1-calibration results;

FIG. 9A is a graph showing pure quadratic calibration results;

FIG. 9B is a graph showing pure quadratic delecte-1-calibration results;

FIG. 10 is a baseline corrected ATR spectra of aqueous glucose solutions;

FIG. 11A is a graph showing multiple linear regression calibration, linear fit, results;

FIG. 11B is a graph showing multiple linear regression calibration, quadratic fit, results;

FIG. 12A is a graph showing multiple linear regression calibration, linear fit, results;

FIG. 12B is a graph showing multiple linear regression calibration, quadratic fit, results;

FIG. 13A is a graph showing multivariate calibration results;

FIG. 13B is a graph showing multivariate cross validation results;

FIG. 14 is a baseline corrected ATR spectra of an LRS glucose solution;

FIG. 15A is a graph showing multiple linear regression calibration, linear fit, results;

FIG. 15B is a graph showing multiple linear regression calibration, quadratic fit, results;

FIG. 16A is a graph showing multiple linear regression calibration, linear fit, results;

FIG. 16B is a graph showing multiple linear regression calibration, quadratic fit, results;

FIG. 17A is a graph showing multivariate calibration results;

FIG. 17B is a graph showing cross validation results;

FIG. 18 is a baseline corrected ATR spectra of a number of CFC fluid sample collected from pre and post diabetic rats;

FIG. 19A is a graph showing multiple linear regression calibration in CFC fluid, linear fit, results;

FIG. 19B is a graph showing multiple linear regression calibration in CFC fluid, quadratic fit, results;

FIG. 20A is a graph showing multiple linear regression calibration in CFC fluid, linear fit, results;

FIG. 20B is a graph showing multiple linear regression calibration in CFC fluid, quadratic fit, results;

FIG. 21A is a graph showing multivariate calibration results;

FIG. 21B is a graph showing multivariate cross-validation results;

FIG. 22 is a baseline corrected ATR spectra of human serum samples with known but varied quantities of glucose added thereto;

FIG. 23A is a graph showing multiple linear regression calibration in human serum, linear fit, results; and

FIG. 23B is a graph showing multiple linear regression calibration in human serum, quadratic fit, results.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

While the invention is susceptible to various modifications and alternative forms, a specific embodiment thereof has been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that there is no intent to limit the invention to the particular form disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

Organic substances can influence electromagnetic radiation. For example, when electromagnetic radiation encounters an organic substance the radiation can be absorbed or transmitted, depending upon the nature of the organic molecules it encounters. If the electromagnetic radiation is absorbed, then the absorption gives rise to absorption bands at particular wavelength regions of an absorption spectrum of the organic substance. (Note that examples of ways to express wavelength regions include, but are not limited to, frequency, wavelength, or wavenumber.)

With respect to coherent or incoherent infrared electromagnetic radiation, it should be understood that, as discussed in greater detail below, an organic substance has an infrared absorption spectrum which includes a set (n) of wavelength regions with each wavelength region corresponding to an absorption band of the absorption spectrum. For example, FIG. 1 shows the infrared absorption spectrum of distilled water alone (see curve A) and 0.5% glucose in distilled water (see curve B). FIG. 1 also shows the infrared absorption spectrum of lactated ringers solution (LRS) alone (see curve C) and 0.5% glucose in LRS (see curve D). Note that LRS contains lactate, sodium, potassium, calcium, and chloride ions and is used, for example, in the rehydration of animals in an emergency. LRS is utilized herein to mimic various biological fluids, including, but not limited to, capillary filtrate collector fluid. Other clinical biological fluids the methods described herein can be utilized with include, but are not limited to, saliva, tears, and urine, and blood components, such as plasma. However, it should be understood that the methods described herein are not limited to just biological samples, such as biological fluids, but can be utilized to measure the amount of an organic substance in other various types of samples.

As shown in FIG. 1, absorption of the infrared electromagnetic radiation by glucose is seen in the about 1200 cm^{-1} to about 950 cm^{-1} range of the spectrum. In particular, the infrared absorption spectrum of FIG. 1 shows absorption bands centered at about 1107 cm^{-1}, about 1080 cm^{-1}, about 1035 cm^{-1}, about 1150 cm^{-1}, and about 993 cm^{-1} for glucose dissolved in distilled water and for glucose dissolved in LRS. Accordingly, glucose has a set (n) of absorption bands within the about 1200 cm^{-1} to about 950 cm^{-1} range of the spectrum. In this case (n) equals 5, i.e., an absorption band centered at about 1107 cm^{-1}, about 1080 cm^{-1}, about 1035 cm^{-1}, about 1150 cm^{-1}, and about 993 cm^{-1}. It should be appreciated that, as shown in FIG. 1, at least a portion of each absorption band occurs between two selected wavenumbers. For example, the absorption band centered at about 1107 cm^{-1} occurs between wavenumbers about 1094 cm^{-1} and about 1118 cm^{-1}. It should be understood that the area of the spectrum between two wavenumbers, at which an absorption band occurs, is referred to herein as a wavelength region. As such, each absorption band has a substantially corresponding selected wavelength region. For example, the absorption band at about 1107 cm^{-1} has a substantially corresponding wavelength region of about 1094 cm^{-1} to about 1118 cm^{-1}. In a similar manner, the absorption

band at about 1080 cm^{-1} has a substantially corresponding wavelength region of about 1075 cm^{-1} to about 1090 cm^{-1}. The absorption band at about 1035 cm^{-1} has a substantially corresponding wavelength region of about 1018 cm^{-1} to about 1048 cm^{-1}. The absorption band at about 1150 cm^{-1} has a substantially corresponding wavelength region of about 1137 cm^{-1} to about 1175 cm^{-1}. The absorption band at about 993 cm^{-1} has a substantially corresponding wavelength region of about 983 cm^{-1} to about 1003 cm^{-1}. Therefore, it should be appreciated that each absorption spectrum also has a set (n) of wavelength regions, and since each absorption band has a substantially corresponding wavelength region, the set (n) of wavelength regions equals the set (n) of absorption bands. For example, with respect to the absorption spectrum of glucose shown in FIG. 1, the set (n) of absorption bands equals 5 and accordingly the set (n) of wavelength regions also equals 5.

As discussed above, the glucose absorption spectrum of FIG. 1 has 5 absorption bands respectively centered at about 1107 cm^{-1}, about 1080 cm^{-1}, about 1035 cm^{-1}, about 1150 cm^{-1}, and about 993 cm^{-1}. It should be appreciated that glucose not only absorbs infrared electromagnetic radiation at each particular wavenumber mentioned above, but also at higher and lower wavenumbers around each of the aforementioned centered wavenumbers. In other words, an absorption band of an organic substance (e.g., glucose) has a width, and therefore will absorb a range or a wavelength band of infrared electromagnetic radiation. As discussed above, a wavelength region is the area of the spectrum between two wavenumbers at which an absorption band occurs. Accordingly, a wavelength band is the range of wavenumbers (or other methods of measuring electromagnetic radiation including, but not limited to, frequency or wavelengths) within a wavelength region at which an organic substance absorbs electromagnetic radiation. In other words, each wavelength band substantially corresponds to a wavelength region. For example, as previously mentioned, the absorption spectrum of glucose shown in FIG. 1, has 5 wavelength regions, i.e., (i) about 1094 cm^{-1} to about 1118 cm^{-1}, (ii) about 1075 cm^{-1} to about 1090 cm^{-1}, (iii) about 1018 cm^{-1} to about 1048 cm^{-1}, (iv) about 1137 cm^{-1} to about 1175 cm^{-1}, and (v) about 983 cm^{-1} to about 1003 cm^{-1}. Therefore, the absorption spectrum of glucose shown in FIG. 1 also has 5 substantially corresponding wavelength bands at which glucose absorbs the electromagnetic radiation, i.e., (i) about 1094 cm^{-1} to about 1118 cm^{-1}, (ii) about 1075 cm^{-1} to about

1090 cm^{-1}, (iii) about 1018 cm^{-1} to about 1048 cm^{-1}, (iv) about 1137 cm^{-1} to about 1175 cm^{-1}, and (v) about 983 cm^{-1} to about 1003 cm^{-1}. However, note that a wavelength region and a wavelength band do not necessarily have to be a range if the organic substance of interest and the nature of the electromagnetic radiation is such that a single wavenumber (frequency or wavelength) can be utilized in the methods described herein. Therefore, as used herein, the terms "wavelength region" and "wavelength band" can be a range or can consist of a single wavenumber (frequency or wavelength). It should also be understood that, while examples of the methods described herein utilize incoherent infrared electromagnetic radiation, coherent infrared electromagnetic radiation can also be utilized.

Still referring to FIG. 1, it should be understood that the glucose absorption spectrum shown therein has a number of reference wavelength bands as well. A reference wavelength band is similar to one of the above described wavelength bands, however in contrast to a wavelength band, a reference wavelength band is a range of wavenumbers at which (i) the organic substance of interest does not substantially influence the electromagnetic radiation but other compounds present within the biological sample do influence the electromagnetic radiation or (ii) no organic substance present within the biological sample substantially influences the electromagnetic radiation. For example, a range of wavenumbers at which the organic substance of interest does not substantially absorb infrared electromagnetic radiation, while other organic substances present in the biological sample do absorb infrared electromagnetic radiation, can be utilized as a reference wavelength band in the methods described herein. In addition, a range of wavenumbers at which no organic substance present in the biological fluid substantially absorbs infrared electromagnetic radiation can be utilized as a reference wavelength band in the methods described herein. In particular, the organic substance of interest with respect to the absorption spectrum shown in FIG. 1 is glucose, accordingly potential wavenumber ranges which can serve as reference wavelength bands are (i) those wavenumber ranges at which glucose substantially does not absorb the infrared electromagnetic radiation while other compounds present in the sample do absorb the infrared electromagnetic radiation and (ii) those wavenumber ranges at which no organic substance substantially absorbs the infrared electromagnetic radiation. Preferably, a reference wavelength band is selected where no organic substance

substantially influences or absorbs the electromagnetic radiation, however this is not necessary for the performance of the methods described herein.

Selecting a reference wavelength band having the above described characteristics allows the spectral data (e.g., absorbance) obtained from detecting electromagnetic radiation within a particular reference wavelength band to be utilized as a baseline measurement. This baseline measurement data is also processed with the aforementioned mathematical model to obtain a measurement of the amount of the organic substance of interest present within the biological sample. Two specific examples of reference wavelength bands are shown in FIG. 1, i.e., one at about 1090 cm^{-1} to about 1095 cm^{-1} and another one at about 1170 cm^{-1} to about 1180 cm^{-1}.

To provide a measurement of the amount (e.g., the concentration) of an organic substance contained within a biological sample, the biological sample is illuminated with electromagnetic radiation, such as infrared electromagnetic radiation. For example, a beam of incoherent infrared electromagnetic radiation can be passed through a biological fluid, such as capillary filtrate fluid, so that the organic substance of interest contained within the biological fluid influences the electromagnetic radiation. Preferably, the organic substance contained within the biological fluid absorbs the electromagnetic radiation so as to create an absorption spectrum which, as discussed above, includes a set (n) of wavelength regions where each of the wavelength regions substantially correspond to an absorption band of the absorption spectrum. After illuminating the biological sample with the electromagnetic radiation, the intensity (e.g., detecting the transmittance) of the wavelength bands and reference wavelength bands are detected with a detection system. In particular, it should be understood that the intensity of only the wavelength bands and the reference bands are detected with the detection system. Furthermore, it should be understood that not all of the wavelength bands and reference wavelength bands are detected. In particular, only a select number of wavelength bands of the absorption spectrum are detected along with only a select number of reference wavelength bands. Therefore, it should be appreciated that only the selected wavelength bands and reference wavelength bands are detected with the detection system while the rest of the electromagnetic radiation is substantially prevented from being detected by the detection system. For example, the electromagnetic radiation not included in the selected wavelength bands and reference wavelength bands can be substantially

filtered out prior to reaching the detection system. In other words, the detection of the wavelength bands and reference wavelength bands is restricted to a select number of wavelength bands of electromagnetic radiation and a select number of reference wavelength bands of electromagnetic radiation. In particular, the number of selected wavelength bands of electromagnetic radiation is equal to n-1 or less. That is, the number of selected wavelength bands of electromagnetic radiation is a subset of (n).

With respect to which particular wavelength band, or combination of wavelength bands, is/are selected for detection is dependent upon which wavelength band(s), in combination with the selected reference wavelength band(s), yields spectral data for processing with a mathematical model so as to provide a useful measurement of the amount of organic substance contained within the biological sample. What is meant herein by "useful" measurement is that the measurement of the amount of organic substance contained with the biological sample is accurate and/or precise enough such that it would be acceptable to utilize in a particular measurement, assay, or application. For example, if a method described herein is utilized in providing a measurement of the amount of glucose contained within capillary filtrate fluid of a diabetic patient, the wavelength band(s) and reference wavelength band(s) must be selected so that the spectral data supplied to the mathematical model from the combination of these bands results in a glucose measurement that is accurate and/or precise enough such that it informs the patient as to his or her glucose levels within acceptable limits.

Factors to consider when selecting which wavelength band(s) to detect include for example (i) ensuring that the absorption band contained within the wavelength band is, or includes, an absorption band of the organic substance of interest, (ii) selecting a wavelength band which has relatively strong absorption, and (iii) selecting a wavelength band where the strength of the wavelength band correlates well with the amount of organic substance of interest contained in the biological sample. In addition, it is preferable that the selected wavelength band(s) is relatively free of interference from absorption bands caused by substances other than the organic substance of interest present in the sample (e.g., the selected wavelength band is separated from the wavelength band of the potentially interfering substance). However, it should be understood that in order to utilize the methods described herein, the selected wavelength band(s) does not have to be free of interfering

absorption bands caused by substances other than the organic substance of interest. Accordingly, a selected wavelength band(s) may be relatively free of interference from absorption bands caused by substances other than the organic substance of interest, or the selected wavelength band(s) may include interfering absorption bands caused by substances other than the organic substance of interest. Therefore, it should be appreciated that the selected wavelength band(s) can (i) be relatively free of interference from absorption bands caused by substances other than the organic substance of interest present in the sample, (ii) include interfering absorption bands caused by substances other than the organic substance of interest present in the sample, or (iii) be a combination of selected wavelength bands in which some are relatively free of interference from absorption bands caused by substances other than the organic substance of interest while others include interfering absorption bands caused by substances other than the organic substance of interest.

Furthermore, the particular mathematical model (e.g., algorithm) and wavelength band(s) and reference wavelength band(s) selected for a particular sensor configuration (discussed below) are determined by the performance of the calibration procedure (discussed below) used for a specific biological sample. Moreover, in order to obtain a useful measurement from a particular sensor configuration, the mathematical model, selected wavelength band(s), and selected reference wavelength band(s) may differ depending upon the nature of the biological sample the organic substance of interest is contained within. For example, different selected wavelength band(s) and selected reference wavelength band(s), in addition to a different mathematical model may be needed depending upon whether the organic substance is contained within for example, plasma or capillary filtrate fluid. Additional factors to consider are measurement accuracy requirements and the economics of the electromagnetic radiation source, optical filters, and detection elements. It should be appreciated that each of the aforementioned factors for a particular application of the methods described herein can be determined by one of ordinary skill in the art by routine experimentation.

After identifying wavelength bands that meet one or more of the aforementioned criteria, which wavelength bands are actually selected for detection and utilization in one of the methods described herein is determined. In particular, different combinations of wavelength bands, or a single wavelength band, along with

one or more reference wavelength bands are utilized until it is determined which combination yields a useful measurement.

The detection system generates an electrical signal as a result of detecting the intensity of the selected wavelength band(s) and reference wavelength band(s). The electrical signal is processed to yield data which is utilized to provide a useful measurement of the amount of the organic substance of interest contained within the biological sample. For example, data generated by the electrical signal can be processed by a mathematical model, such as a quantification algorithm, so as to provide a useful measurement of the amount of the organic substance of interest contained within the biological sample.

It should be appreciated that detecting and processing spectral data only from the selected wavelength band(s) and reference wavelength band(s) simplifies the process of providing a useful measurement of the amount of an organic substance of interest contained within a biological sample. For example, since an apparatus for performing a method described herein only detects and processes spectral data from a select number of wavelength bands and reference wavelength bands it is less complex as compared to an apparatus configured to detect and process spectral data from all of the wavelength bands of an absorption spectrum. Accordingly, an apparatus configured to perform one of the methods described herein lends itself to being smaller, compact and portable.

While the above description is directed to the preferred method of limiting the detection to select wavelength bands and reference wavelength bands, an alternative embodiment of a method for measuring an amount of an organic substance contained within a biological sample is to detect all of the wavelength bands, but only process the spectral data from the aforementioned selected wavelength bands with the mathematical model.

Now referring to FIG. 2, there is shown an example of a fiber optic evanescent wave sensor 10 which can be utilized in the methods described herein. In particular, the spectral data obtained from this type of sensor 10 can be utilized in the quantification of an organic substance contained in a biological fluid. A fiber optic evanescent wave sensor of the type shown in FIG. 2 is described by Karlowatz, M., Kraft, M., Eitenberger, E., Mizaikoff, B. and Katzir, A. in "Chemically Tapered Silver Halide Fibers: An Approach for Increasing the Sensitivity of Mid-Infrared

Evanescent Wave Sensors," *Applied Spectroscopy*, 54(11), pp. 1629-1633 (2000), which is incorporated herein by reference. Another fiber optic evanescent wave sensor of the type shown in FIG. 2 is described by Han, L., Lucas, D., Littlejohn, D., and Kyauk, S. in "NIR Fiber-Optic Method with Multivariate Calibration Analysis for Determination of Inorganic Compounds in Aqueous Solutions," *Applied Spectroscopy*, 54(10), pp. 1447-1452 (2000), which is also incorporated herein by reference.

Briefly, sensor 10 shown in FIG. 2 includes a number of optic fibers, for example sensor 10 can include an optical fiber bundle 18 having a coupler 20 which splits into optical fibers 22, 24, and 26. The number of optical fibers sensor 10 includes is determined by the number of selected wavelength bands and reference wavelength bands utilized in determining the amount of the organic substance contained in the biological fluid. For example, sensor 10 includes 3 optical fibers, one for a first selected wavelength band, one for a second selected wavelength band, and one for a reference wavelength band. Sensor 10 also includes a modulated infrared source 12, a regulated power supply 14, and focusing optics 16 which focuses the modulated infrared beam into optical fiber bundle 18 and thus into optical fibers 22, 24, and 26. Sensor 10 further includes filters 36, a detection system 52 having a number of detection elements 38, a mode-lock amplifier 40, and a signal processor 42.

Modulated infrared source 12 and signal processor 42 are electrically coupled to mode-lock amplifier 42 via electrical lines 44 and 46, respectively. In addition, each detection element 38 is electrically coupled to mode-lock amplifier 42 via an electrical line 48 and an electrical line 50. A receiving end 30 of each optical fiber 22, 24, and 26 is operatively coupled to a filter 36, which in turn is operatively coupled to a detection element 38.

Note that optic fibers 22, 24, and 26 are unclad through a portion of their length where biological fluid 34 flows over the fiber. The length of this portion is determined by the signal-to-noise ratio requirements. In sensor 10 the transmitting and receiving ends are continuous, i.e., made of the same fibers. The attenuation or absorbance of electromagnetic radiation advanced through optic fibers 22, 24, and 26 due to the organic substances contained within biological fluid 34 takes place via the aforementioned evanescent wave phenomenon. Note that filters 36 are optical

bandpass filters that limit the range of wavelengths of electromagnetic radiation which pass therethrough. In particular, filters 36 are configured so that only the select wavelength bands and the select reference wavelength band are allowed to substantially pass therethrough and thus be detected by detection elements 38.

When operating sensor 10 power supply 14 provides power to infrared source 12 such that infrared source 12 generates a beam of incoherent infrared electromagnetic radiation directed toward focusing optics 16. Focusing optics 16 focuses the radiation onto coupler 20 which in turn directs the radiation through fiber bundle 18. Specifically, the radiation is transmitted through optic fibers 22, 24, and 26, and thus pass through biological fluid 34 as biological fluid 34 is advanced through a sample cell 84 in the direction indicated by arrow 86. Certain wavelengths of the radiation are absorbed by organic substances contained within biological fluid 34 as the radiation passes therethrough. After passing through biological fluid 34 the radiation interacts with filters 36. As discussed above, filters 36 restrict the infrared electromagnetic radiation allowed to substantially pass therethrough to the selected wavelength bands and the selected reference wavelength band. Each selected wavelength band and the selected reference wavelength band interacts with a detection element 38. Each detection element 38 generates an electrical signal in response to interacting with a wavelength band or the reference wavelength band. The electrical signal is communicated to mode-lock amplifier 40 via the aforementioned electrical lines. Each electrical signal is then communicated to processor 42 (such as an integrated circuit) via electrical line 46. Processor 42 then processes the electrical signals with a mathematical model, such as a quantification algorithm, so as to provide a useful measurement of the amount of the organic substance of interest (e.g., glucose) contained within biological fluid 34.

Now referring to FIG. 3, there is shown an example of an attenuated total reflection (ATR) fiber optic evanescent wave sensor 54 which can be utilized in the methods described herein. Sensor 54 is substantially similar to sensor 10 described above and thus the same reference numbers are used to indicate the corresponding components. In addition, since sensor 54 is similar to sensor 10 and operates in a similar manner, only substantial differences between sensor 54 and sensor 10 are briefly discussed herein.

As previously mentioned, the construction of sensor 54 is similar to sensor 10 except that transmitting ends 28 and receiving ends 30 of fibers 22, 24, and 26 are separate units and they both terminate at an ATR crystal 56 operatively coupled to one end thereof. Fluid 34 is in contact with each ATR crystal surface 58 but does not come in contact with optical fibers 22, 24, and 26 as fluid 34 flows past ATR crystal surfaces 58 in the direction indicated by arrow 82.

Sensor 54 is also based on the evanescent wave principle mentioned above. However, with sensor 54 the evanescent wave penetrates into fluid 34 via ATR crystals 56 which are kept in contact with fluid 34. The electrical signals are generated and processed in a manner similar to that discussed above in reference to sensor 10.

Now referring to FIG. 4, there is shown an example of a fiber optic injection transmission sensor 60 which can be utilized in the methods described herein. Sensor 60 is substantially similar to sensor 10 described above and thus the same reference numbers are used to indicate the corresponding components. In addition, since sensor 60 is similar to sensor 10 and operates in a similar manner, only substantial differences between sensor 60 and sensor 10 are briefly discussed herein.

A fiber optic fluid injection transmission sensor of the type shown in FIG. 4 is described by Lendl, B., Schindler, R., Frank, J., and Keilner, R. (1997), in "Fourier Transform Infrared Detection in Miniaturized Total Analysis Systems for Sucrose Analysis," Analytical Chemistry, 69(15), pp. 2877-2881 (1997) which is incorporated herein by reference. Briefly, transmitting ends 28 and receiving ends 30 of optical fibers 22, 24, and 26 terminate at infrared transmitting windows 62 which are positioned tens of microns apart. Transmitting windows 62 define a biological sample cell 64 (for example about 10 microns wide). Biological fluid 34 is positioned within, or flows through (in the direction indicated by arrow 80), sample cell 64 which defines the pathlength for the infrared absorption.

Now referring to FIG. 5A, there is shown another example of a sensor 66 which can be utilized in the methods described herein. Sensor 66 includes a miniature pulsable infrared emitter source 68 with a parabolic reflector. An example of a miniature pulsable infrared emitter source 68 which can be utilized with the methods described herein is commercially available from Ion Optics, which is located in Waltham, Massachusetts. Source 68 includes an electrically coupled infrared

source power supply and modulator circuit 74. An example of a power supply and modulator circuit 74 which can be utilized with the methods described herein is commercially available from Boston Electronics, located in Brookline, Massachusetts. Source 68 also includes a multi-channel miniature pyroelectric infrared detector 70. Detector 70 includes an electrically coupled pyroelectric detector preamplifier and signal processing circuit 76. An example of a multi-channel miniature pyroelectric infrared detector 70 which can be utilized with the methods described herein is commercially available from InfraTec GmbH, located in Dresden, Germany. Additional infrared detectors which can be utilized in the methods described herein are commercially available from Wilks Enterprise, Inc. located in South Norwalk, Connecticut. Sensor 66 further includes a biological sample cell 78 interposed between source 68 and detector 70. Cell 78 has a sample space 72 defined therein so that a biological fluid can be advanced therethrough in the direction indicated by arrow 88. For example, a biological fluid such as capillary filtrate collected from a capillary filtrate collector. One capillary filtrate collector which can be utilized in the methods described herein is described by Ash S.R. et al. in "Subcutaneous Ultrafiltration Fibers for Chemical Sampling of Blood: The Capillary Filtrate Collector (CFC)" in Leung WW-F. ed. Proceedings of the National Meeting of the American Filtration Society. Chicago : Advances in Filtration and Separation Technology, Vol. 7, 1993:316-319, which is incorporated herein by reference.

During operation of sensor 66, source 68 generates a low frequency infrared electromagnetic radiation pulse. Circuit 74 is configured to optimize the signal-to-noise ratio of the pulse reaching the biological fluid contained within sample space 72. The radiation is transmitted through sample space 72 and thus passes through the biological fluid contained therein. As previously discussed, certain wavelengths of the radiation are absorbed by organic substances contained within the biological fluid 34 as the radiation passes therethrough. The radiation then interacts with detector 70 which is configured so that only the select wavelength bands and the select reference wavelength band are substantially detected by detector 70. Upon detecting the select wavelength bands and the select reference wavelength band an electrical signal is sent to circuit 76 which processes the electrical signal with a mathematical model to provide a useful measurement of the amount of the organic substance of interest contained within the biological fluid. Note that circuit 76 has a

frequency synchronization connection 90 that ensures that high signal-to-noise ratios are maintained through modulated signal detection.

Now referring to Fig. 5B, there is shown still another example of a sensor 92 which can be utilized in the methods described herein. In particular, sensor 92 is a reflection-absorption based infrared sensor. Briefly sensor 92 includes a base 94 having a receptacle area 110 defined therein. Receptacle area 110 has a floor 114 with a reflective surface 116 defined thereon. Sensor 92 also includes a regulated power supply 96 operatively coupled to base 94. Sensor 92 further includes a modulated infrared source 98 and focusing optics 100. Modulated infrared source 98 is operatively coupled to power supply 96 and focusing optics 100. In addition, modulated infrared source 98 and focusing optics 100 are positioned relative to receptacle area 110 such that infrared electromagnetic radiation generated by modulated infrared source 98 is directed onto receptacle area 110 by focusing optics 100. Sensor 92 also includes a detection element 102 operatively coupled to a filter assembly 108. Detection element 102 is operatively coupled to base 94 such that infrared electromagnetic radiation reflected off of reflective surface 116 impinges onto filter assembly 108 and detection element 102. Sensor 92 further includes a mode-lock amplifier 106 which is operatively coupled to detection element 102 and power supply 96 via electrical lines 104 and electrical line 118, respectively.

When operating sensor 92 power supply 96 provides power to infrared source 98 such that infrared source 98 generates a beam of infrared electromagnetic radiation directed toward focusing optics 100. Focusing optics 100 directs the radiation through a fluid film 112 positioned within receptacle area 110. The radiation is transmitted through fluid film 112 and is reflected off of reflective surface 110 so that it interacts with filter assembly 108. As discussed above, certain wavelengths of the radiation are absorbed by organic substances contained within fluid film 112 as the radiation passes therethrough. As discussed above, filter assembly 108 restricts the infrared electromagnetic radiation allowed to substantially pass therethrough to the selected wavelength bands and the selected reference wavelength band. Each selected wavelength band and the selected reference wavelength band interacts with a detection element 102 which generates an electrical signal in response to interacting with a wavelength band or the reference wavelength band. The electrical signal is communicated to mode-lock amplifier 106 via the

aforementioned electrical lines. Each electrical signal is then communicated to a processor (such as an integrated circuit; not shown) via an electrical line (not shown). The processor then processes the electrical signals with a mathematical model, such as a quantification algorithm, so as to provide a useful measurement of the amount of the organic substance of interest (e.g., glucose) contained within fluid film 112.

Now referring to FIG. 5C there is shown an example of a miniature ATR sensor 120 which can be utilized in the methods described herein. Sensor 120 includes a base 122, a regulated power supply 124 operatively coupled to base 122 and a detector signal conditioning and amplification circuit 126 also operatively coupled to base 122. Sensor 120 further includes an infrared source 126 and focusing optics 128 operatively coupled to power supply 124. Infrared source 126 and focusing optics 128 are positioned relative to an ATR crystal 130 so that infrared electromagnetic radiation generated by infrared source 126 is directed through ATR crystal 130 and into a sample 132 by focusing optics 128. Sensor 120 also includes a detection element 134, such as a multichannel detector, operatively coupled to a filter assembly 136. Detection element 134 is positioned relative to ATR crystal 130 such that infrared electromagnetic radiation being emitted through ATR crystal 130 impinges onto filter assembly 136 and detection element 134. Sensor 120 further includes a mode-lock amplifier 140 which is operatively coupled to detector signal conditioning and amplification circuit 126 and power supply 124 via electrical lines 138 and electrical line 142, respectively.

When operating sensor 120 power supply 124 provides power to infrared source 126 such that infrared source 126 generates a beam of infrared electromagnetic radiation directed toward focusing optics 128. Focusing optics 128 directs the radiation through sample 132 positioned in contact with ATR crystal 130. As discussed above, certain wavelengths of the radiation are absorbed by organic substances contained within sample 132 as the radiation passes therethrough. The radiation exits the ATR crystal 130 positioned in contact with sample 132 and interacts with filter assembly 136. Filter assembly 136 restricts the infrared electromagnetic radiation allowed to substantially pass therethrough to the selected wavelength bands and the selected reference wavelength band. Each selected wavelength band and one or more selected reference wavelength bands interact with detection element 134 which generates an electrical signal in response to interacting

with a wavelength band or the reference wavelength band. The electrical signal is communicated to mode-lock amplifier 140 via the aforementioned electrical lines. Each electrical signal is then communicated to a processor (such as an integrated circuit; not shown) via an electrical line (not shown). The processor then processes the electrical signals with a mathematical model, such as a quantification algorithm, so as to provide a useful measurement of the amount of the organic substance of interest contained within sample 132. (Note that it is estimated that 10 – 20 reflections will provide adequate and measurable infrared absorption due to the organic substances in the biological sample.)

The following example illustrates utilizing one wavelength band and one reference wavelength band for providing a measurement of the amount of glucose contained within LRS. In particular, eight known concentrations of glucose dissolved in LRS were prepared, i.e., 0%, 0.05%, 0.075%, 0.1%, 0.2%, 0.3% 0.4%, and 0.5% glucose. The selected wavelength band for this example is the range of wavenumbers from about 1075 cm^{-1} to about 1090 cm^{-1} (see FIG. 1). The selected reference wavelength band for this example is the range of wavenumbers from about 1090 cm^{-1} to about 1095 cm^{-1} (see FIG. 1). Each glucose solution was illuminated with incoherent electromagnetic radiation and the absorption band corresponding to the selected wavelength band and the selected reference wavelength band was measured. In addition, the absorption band corresponding to the wavelength band, i.e., the wavenumbers from about 1075 cm^{-1} to about 1090 cm^{-1} for each glucose concentration was integrated. Furthermore, the absorption band corresponding to the reference wavelength band, i.e., the wavenumbers from about 1090 cm^{-1} to about 1095 cm^{-1} for each glucose concentration was also integrated. Note that the integrated absorbances were divided by their corresponding band widths to avoid scaling effects. Thereafter, a mean-centered integrated absorbance band ratio for the wavelength band and the reference wavelength band was calculated for each glucose concentration. In particular, the absorbance band ratio was calculated by dividing the mean-centered integration value for the absorbance of the selected wavelength band by the mean-centered integration value for the absorbance of the selected reference wavelength band. Accordingly, each of the above described glucose concentrations has a mean-centered integrated absorbance ratio associated with it as shown in Table 1 below.

TABLE 1

Glucose Concentration	Integrated Absorbance Ratio
0%	0.896246
0.05%	0.896550
0.075%	0.897490
0.1%	0.897669
0.2%	0.899011
0.3%	0.901061
0.4%	0.902709
0.5%	0.905265

* The above values are not mean-centered. Mean-centering is done by the Matlab code discussed below.

The above mean-centered values of the glucose concentrations and the integrated absorbance ratios were used to generate a regression model and to obtain calibration constants. The concentration of glucose in solution was calibrated using the following equation 1:

$$C_g = P_0 + P_1 IAR_{\lambda,1} + P_2 IAR^2_{\lambda,1} \quad \text{(equ. 1)}$$

where (i) C_g is the mean-centered concentration of glucose in the sample measured using methods other than IR absorption, (ii) P_i are calibration constants, and (iii) $IAR_{\lambda,1}$ is a mean-centered integrated absorbance ratio for the selected wavelength band and selected reference wavelength band. As previously mentioned, in this equation the variables are mean-centered. The values of the calibration constants are calculated by Matlab 6.1.0.450 release 12.1, the MathWorks Inc. utilizing the following code:

```
clc;
pwd
fid1=fopen('MLR_glucose.tst');
n=7
[C,count_C]=fscanf(fid1,'%g',[1,n]);
[A,count_A]=fscan(fid1,%g',[n,2]);
A
C=C'
mA-mean(a)
mC=mean(C)
B=A(:,1)-mA(:,1)
B1=A(:,2)-mA(:,2)
mcC=C-mC
mcA-cat(2,B,B1)
Regstats(mcC,mcA,'quadratic')
```

This code mean centers the concentration and absorbance and does a multiple linear regression on the given absorbance matrix and concentration vector (quadratic uses constant, linear, crossproduct and square terms). Note that the values of the calibration constants are used in the validation. Further note that validation of the calibration constants is done by reworking the calibration after deleting 1 point. The resulting fit is used to predict the deleted point. The results of the computations utilizing the Matlab program to process the aforementioned data are shown below in Table 2.

TABLE 2

Actual % Glucose	Calibration %	Validation %
0	0.0074	0.0019
0.05	0.0287	0.0388
0.075	0.0928	0.0888
0.1	0.1046	0.1036
0.2	0.1897	0.1935
0.3	0.3075	0.3029
0.4	0.3915	0.3958
0.5	0.5028	0.4770

*Validation is done by delete-1-calibration.

Both linear (using only the first two terms in the above equation) and quadratic (using all the terms in the above equation) regression fits were considered. The quadratic fit of the integrated absorbance bands gave the best results for the LRS glucose solutions using this model. The resultant fits of the actual and measured glucose concentrations are shown in FIGS. 6A and 6B. Accordingly, it should be appreciated that a sensor, such as one of the sensors described herein, can utilize calibration constants obtained in the above described manner and give a useful measurement of the glucose concentration in a biological sample based upon the absorbance signal from the wavelength band and the reference wavelength band.

The above described procedure was repeated in a substantially identical manner with the exception that aqueous glucose solutions were prepared rather than LRS glucose solutions. Both linear and quadratic regression fits were considered for the aqueous glucose solutions. The quadratic fit of the integrated absorbance bands gave the best results for the aqueous glucose solutions using the above described model. The resultant fits of the actual and measured aqueous glucose concentrations are shown in FIGS. 7A and 7B.

The following is another example which illustrates utilizing a method described herein to provide a measurement of the amount of glucose contained within

an LRS solution. However, in this method the absorbance from two wavelength bands and one reference wavelength band are utilized for providing a measurement of the amount of glucose contained within the LRS solution. The method is similar to that described above for utilizing one wavelength band and one reference wavelength band. In particular, eight known concentrations of glucose dissolved in LRS were prepared, i.e., 0%, 0.05%, 0.075%, 0.1%, 0.2%, 0.3% 0.4%, and 0.5% glucose. The selected wavelength bands for this example are the range of wavenumbers from about 1075 cm^{-1} to about 1090 cm^{-1} (see FIG. 1) and the range of wavenumbers from about 1137 cm^{-1} to about 1175 cm^{-1}. The selected reference wavelength band for this example is the range of wavenumbers from about 1170 cm^{-1} to about 1180 cm^{-1} (see FIG. 1). As described above, each glucose solution was illuminated with incoherent electromagnetic radiation and the absorption bands corresponding to the selected wavelength bands and the selected reference wavelength band was measured. In addition, the absorption band corresponding to the wavelength bands, i.e., the wavenumbers from about 1075 cm^{-1} to about 1090 cm^{-1} and the wavenumbers from about 1137 cm^{-1} to about 1175 cm^{-1}, for each glucose concentration was integrated. Furthermore, the absorption band corresponding to the reference wavelength band, i.e., the wavenumbers from about 1170 cm^{-1} to about 1180 cm^{-1} for each glucose concentration was also integrated. Note that, as before, the integrated absorbances were divided by their corresponding band widths to avoid scaling effects. Thereafter, a mean-centered integrated absorbance band ratio for each of the wavelength bands and the reference wavelength band was calculated for each glucose concentration. In particular, as before, the absorbance band ratio was calculated by dividing the mean-centered integration value for the absorbance of each of the selected wavelength bands by the mean-centered integration value for the absorbance of the selected reference wavelength band. Accordingly, each of the above described glucose concentrations has a mean-centered integrated absorbance ratio associated with it as shown in Table 3 below.

TABLE 3

Glucose Concentration	Integrated Absorbance Ratio for 1075 cm^{-1}-1090 cm^{-1} wavelength band	Integrated Absorbance Ratio for 1137 cm^{-1}-1175 cm^{-1} wavelength band
0%	0.926683	0.879391
0.05%	0.927934	0.879949
0.075%	0.927893	0.880182
0.1%	0.929363	0.880122
0.2%	0.931147	0.880795
0.3%	0.936557	0.881594
0.4%	0.943733	0.882822
0.5%	0.946844	0.883629

* The above values are not mean-centered. Mean-centering is done by the Matlab code discussed below.

As before, the above mean-centered values of the glucose concentrations and the integrated absorbance ratios were used to generate a regression model and to obtain calibration constants. The concentration of glucose in solution was calibrated utilizing the following equation 2:

$$C_g = P_0 + P_1IAR_{\lambda,1} + P_2IAR_{\lambda,2} + P_3IAR^2_{\lambda,1} + P_4IAR^2_{\lambda,2} + P_5IAR_{\lambda,1}IAR_{\lambda,2} \text{ (equ. 2)}$$

where (i) C_g is the mean-centered concentration of glucose in the sample measured using methods other than IR absorption, (ii) P_i are calibration constants, and (iii) $IAR_{\lambda,j}$ is a mean-centered integrated absorbance ratio of two of the selected infrared wavelength bands and the selected reference wavelength band. As previously mentioned, in this equation the variables are mean-centered. The values of the calibration constants are calculated by Matlab as discussed above utilizing the following code:

```
Clc;
Pwd
Fid1=fopen('MLR_glucose.txt);
N=7
[C,count_C]=fscan(fid1,'%g',[1,n]);
[A,count_A]=fscanf(fid1,'%g',[n,3]);
A
C=C'
mA=mean(A)
mC=mean(C)
B=A(:,1)-mA(:,1)
B1=A(:,2)-mA9(:,2)
B2=A(:,3)-mA(:,3)
mcC=C-mC
mcA=cat(2,B,B1,B2)
regstats(mcC,mcA,'quadratic')
```

As before, this code mean centers the concentration and absorbance and does a multiple linear regression on the given absorbance matrix and concentration vector (quadratic uses constant, linear, crossproduct and square terms). Note that the values of the calibration constants are used in the validation. Further note that validation of the calibration constants is done by reworking the calibration after deleting 1 point. The resulting fit is used to predict the deleted point. The results of the utilizing the Matlab program to process the aforementioned data are shown below in Table 4.

TABLE 4

Actual % Glucose	Calibration %	Validation %
0	0.0016	-0.0683
0.05	0.0526	0.048
0.075	0.0782	0.0705
0.1	0.093	0.0974
0.2	0.1953	0.2031
0.3	0.3075	0.2923
0.4	0.399	0.4327
0.5	0.4997	0.5492

* Validation is done by delete-1-calibration.

Linear, interaction, quadratic, and pure quadratic regression fits were considered. Linear regression involved only the first three terms in the above

equation. Interaction involved the linear terms and the fifth (interaction) term. Quadratic regression involved all the terms in the above equation. Pure quadratic regression involved all the terms except the interaction term. The pure quadratic calibration model for the LRS glucose solutions gave the best results. The resultant fits of the actual and measured glucose concentrations are shown in FIGS. 8A and 8B. Therefore, it should be appreciated that a sensor, such as one of the sensors described herein, can utilize calibration constants obtained in the above described manner and give a useful measurement of the glucose concentration in a biological sample based upon the absorbance signal from two wavelength bands and a single reference wavelength band.

The above described two wavelength band procedure was repeated in a substantially identical manner with the exception that aqueous glucose solutions were prepared rather than LRS glucose solutions. The quadratic fit of the integrated absorbance bands gave the best results for the aqueous glucose solutions using the above described model. The resultant fits of the actual and measured aqueous glucose concentrations are shown in FIGS. 9A and 9B.

It should be appreciated that the presence of lactate in the glucose LRS solutions presents a challenge to the methods described herein since lactate and glucose have absorption bands in the same mid infrared region. However, as demonstrated above, useful correlations can be obtained through the proper selection of wavelength bands, reference wavelength band, and calibration method which can be identified via routine experimentation.

It should be appreciated that while the above examples utilize equations 1 and 2 to calibrate the glucose in the sample solution other equations can be utilized. For example, the following equations 3 and 4 can be utilized:

$$C_g = P_o + P_1 IA_{\lambda,1} + P_2 IA_{\lambda,2} + P_3 IA^2_{\lambda,1} + P_4 IA^2_{\lambda,2} + P_5 IA_{\lambda,1} IA_{\lambda,2} \quad \text{(equ. 3)}$$

where (i) C_g is the mean centered concentration of glucose in solution measured using methods other than IR absorption, (ii) P_i are calibration constants, and (iii) $IA_{\lambda,1}$ and $IA_{\lambda,2}$ are the mean centered integrated absorbance for the selected wavelength band and the selected reference wavelength band,

$$C_g = P_0 + P_1 IA_{\lambda,1} + P_2 IA_{\lambda,2} + P_3 IA_{\lambda,3} + P_4 IA^2_{\lambda,1} + P_5 IA^2_{\lambda,2} + P_6 IA^2_{\lambda,3} + P_7 IA_{\lambda,1} IA_{\lambda,2} + P_8 IA_{\lambda,1} IA_{\lambda,3} + P_9 IA_{\lambda,2} IA_{\lambda,3} \quad \text{(equ. 4)}$$

where (i) C_g is the mean centered concentration of glucose in solution measured using methods other than IR absorption, (ii) P_i are calibration constants, and (iii) $IA_{\lambda,j}$ is the mean centered integrated absorbance for band j.

It should be appreciated that equations 3 and 4 use integrated absorbance rather than integrated absorbance ratios as shown in equations 1 and 2 above, i.e., the reference band is used as additional absorbance terms instead of being used in the denominator term in equations 3 and 4.

The following discussion is directed to obtaining useful measurements of the amount of glucose contained within water, LRS, and CFC fluid (i.e., capillary filtrate collector fluid) utilizing ATR (i.e., Attenuated Total Reflectance) measurements in the mid infrared (about 1200 cm^{-1} to about 900 cm^{-1}) region.

The measurements were made using a Nicolet Nexus7 670 spectrometer equipped for mid infrared measurements with a liquid nitrogen-cooled MCT-A detector and an XT-KBr beamsplitter. Multi-bounce ATR measurements were made by taking the background measurements before each sample measurement. The resolution was set at 4 cm^{-1} and 64 scans were collected and averaged. Using a sealed and dessicated system minimized the atmospheric water vapor and carbon dioxide absorption effects. Further, sufficient period of time was allowed between the mounting of the sample and the actual measurement in order to let the system reach equilibrium.

The aqueous glucose solutions and the LRS solutions were made by dissolving appropriate quantities of d-glucose in distilled water and LRS. The solutions were prepared in large quantities for the lower concentrations and in small quantities for the higher concentrations with a view to maintaining the uncertainties due to weight measurement constant. Concentrations of glucose in these solutions varied in the range of 0.05%-0.5 % or 50-500 mg/dL.

Another fluid obtained for the analysis was from a CFC implanted in a rat's blood stream. The fluid collected was taken and analyzed the same day with minimum refrigeration in between. The amount of fluid obtained in this manner was 1-2 mL. Therefore, a plexiglass insert was designed and used to compress the fluid down to the required volume. This design was useful in wetting the ATR crystal surface without loss of significant amount of fluid. The plexiglass material is

transparent. Therefore, it helped ensure that the crystal was completely wetted via visible inspection. The fluid volume was reduced to 40 μL through this modification.

A total of 52 samples of CFC fluid were obtained in this manner from the same rat on different days. Of these, 9 were rejected due to suspicion of contamination at the implantation site or due to undetectably (using an Accu~chek® meter) low glucose values, 18 samples were from the rat when it remained healthy and 25 were obtained after the rat turned diabetic. After about a month, diabetes was induced in the rat by injection of Streptozotocin. This resulted in the glucose levels reaching higher values than normal and gave a number of calibration points beyond the normal range of 75-150 mg/dL. Values as high as 870 mg/dL were obtained. These values of glucose concentration were measured using an Accu~chek® glucose meter and test strip. Blood glucose levels were monitored as well but since the CFC fluid sample is collected over a period of time, comparisons could not be made between the blood glucose levels and the CFC glucose levels in the present discussion. However, it is known that CFC glucose levels correlate well with blood glucose levels after accounting for the time lag associated with the transport of the CFC fluid from the CFC to the sampling port.

The quantification methods utilized were Inverse Multiple Linear Regression (IMLR) of absorbance bands which correspond to (i) one wavelength band and one reference wavelength band and (ii) two wavelength bands and one reference wavelength band, with linear, interaction and quadratic terms and Partial Least Squares (PLS) considered. In the case of IMLR, as discussed above, mean-centered absorbance values from the selected wavelength band(s) and selected reference wavelength band were used along with the above described ratio of wavelength band(s) absorbance values/ reference wavelength band absorbance values.

2 WAVELENGTH BAND IMLR

In this method, as previously discussed, the integrated absorbance in one wavelength band (e.g., 9.17 um - 9.3 um; 1090 cm^{-1} - 1075 cm^{-1}) and one reference wavelength band (e.g., 9.13 um - 9.17 um; 1095 cm^{-1} - 1090 cm^{-1}) were calculated from the measured spectra of the solutions at different concentrations. As discussed above, the reference wavelength band is used as a reference/baseline band and is used to ratio out the reference signal variations. Further, the integrated

absorbances are divided by the bandwidth to avoid scaling effects. Mean-centered values of the concentrations and integrated absorbance ratios are used to generate a regression model and to obtain the calibration constants. The concentration of glucose in solution was calibrated by using the above discussed equation 1. Both linear (using only the first two terms in equ. 1) and quadratic (using all the terms in equ. 1) regression fits were considered.

3 WAVELENGTH BAND IMLR

This method is similar to the above method except that two wavelength bands and one reference wavelength band were considered as illustrated as an example in Table 1 below. It is noted that the choice of wavelengths utilized in the methods described herein are specific to the type of sample fluid being assayed. For example, the wavelength bands set forth in Table 1 below were selected for glucose in LRS utilizing the criteria described herein (e.g., ensuring that the absorption band contained within the wavelength band is an absorption band of the organic substance of interest, selecting a wavelength band which has relatively strong absorption, and selecting a wavelength band in which absorption is relatively free of interference, or separated from, adjacent absorption bands) . On the other hand, the wavelength bands set forth in Table 2 below were selected for glucose in rat CFC utilizing the criteria described herein. As shown in Table 2, the wavelength bands selected for glucose in rat CFC are different from those selected from glucose in LRS.

Similar to the above described method, i.e., the 2 wavelength band IMLR, mean-centered values of the concentrations and integrated absorbances are used to generate a regression model and to obtain the calibration constants. The concentration of glucose in solution was calibrated using the above discussed equ. 2. Linear, interaction, quadratic and pure quadratic regression methods were considered. Linear regression involved only the first three terms in equ. 2. Interaction involved the linear terms and the fifth (interaction) term. Quadratic regression involved all the terms in equ. 2 and pure quadratic regression involved all the terms except the interaction term.

MULTIVARIATE CALIBRATION USING PLS

Partial Least Squares (PLS) is a multispectral calibration method that uses the absorbance data at many different wavelengths. PLS uses the concentration and absorbance information and represents them in terms of "latent" variables. These latent variables are fitted with a regression equation using a least squares technique. The number of variables is reduced using a principal component analysis (PCA) step. More details of the method can be found in Geladi P. et al. Partial Least-Squares Regression: A Tutorial, Analytica Chimica Acta 1986 1-17, which is incorporated herein by reference.

For the aqueous and LRS glucose solutions, PLS was used in the regions of glucose absorption and no baseline correction was used. In the case of the CFC fluid, a sensitive fit was used and a non-linear fit was used. The best correlations were obtained for a quadratic baseline fit-corrected PLS with a non-linear fit.

It should be appreciated that the above discussed methods are inverse calibration methods. These methods are expected to be more successful in the presence of interferences in signals (absorption) from other components since they do not involve a dependence on the concentrations of the "unimportant" components in the fluid.

AQUEOUS GLUCOSE SOLUTIONS

The ATR spectra of aqueous glucose solutions in the spectral range of about 1200 cm^{-1} to about 975 cm^{-1} (8.3-10.3 μm) are shown in FIG. 10. The major absorption peaks due to glucose are identifiable along with the less prominent ones.

One wavelength band and one reference wavelength band in this region correlate well with glucose concentrations and were selected for the calibration. With respect to the selected reference wavelength band, it was chosen based on its moderately invariant effect with respect to glucose concentration. These selections were based on the best results for the LRS glucose solution spectra discussed below.

The best results for aqueous glucose solutions using the IMLR model discussed above were obtained for quadratic fits. It is well known that Beer's law models are usually linear as the absorbance is expected to vary linearly. However, it

is seen in the present case that the quadratic fits are better probably because the interaction effects are appreciable. FIGS. 11A and 11B show the results of the one wavelength band and one reference wavelength band IMLR with linear and quadratic fits respectively. Both the fits show good performance with a correlation coefficient of 0.999 as is to be expected in the absence of interferences. There is a marginal improvement in the correlation with a quadratic fit.

FIGS. 12A and 12B show the results of the two wavelength band and one reference wavelength band IMLR. The correlation is improved for a quadratic fit. However, it is seen that this fit shows slightly poorer correlation compared to the one wavelength band fit. This may be due to the fact that the reference wavelength band was selected with the LRS interferences to glucose in mind and therefore, the performance worsened in the case of the aqueous glucose solution.

FIGS. 13A and 13B, show the results of the Partial Least Squares calibration (PLS) in the about 1190 cm^{-1} to about 980 cm^{-1} (8.4-10.2 μm) wavenumber range. Since this region contains the glucose absorbance information the correlations are expected to be high. FIG. 13A shows a high correlation coefficient of 0.9999 for the calibration. FIG. 13B shows the results of leave-1-out cross validation, the correlation coefficient being 0.997. These values are close to those obtained in the case of the IMLR methods indicating that most of the glucose absorbance information is contained within the bands chosen for the IMLR analysis.

LRS GLUCOSE SOLUTIONS

LRS was spiked with glucose in the 0.05-0.5% concentration range and the spectra of these solutions in the spectral range of about 1200 cm^{-1} to about 975 cm^{-1} (8.3-10.3 μm) are shown in FIG. 14. The ions (sodium, potassium, calcium and chloride) present in the solution cause a large shift in baseline. These shifts have been accounted for through a baseline correction procedure in FIG. 14. The glucose absorption and the peaks due to lactate are seen. The glucose peaks appear shifted and distorted compared to the ones in the case of aqueous glucose solutions. This is due to the effect of the peaks due to the lactate which are present in the same region as the glucose. The challenge for the IMLR algorithm is therefore, to select the correct wavelengths that correlate with the glucose concentration while accounting for the interfering effects of the lactate. However, it may be argued that there are

systematic effects due to the fact that the concentration of the lactate is expected to correlate with the concentration of glucose. This argument will be settled by the results obtained for the case of CFC fluid presented in the next section.

FIGS. 15A and 15B show the results of a one wavelength band and one reference wavelength band IMLR with linear and quadratic fits respectively. The correlations are down to 0.98 for the linear fit due to the presence of the lactate interferences. However, this is improved to 0.99 for the quadratic fit as the interaction effects are being accounted for.

FIGS. 16A and 16B show the results of a two wavelength band and a one reference wavelength band IMLR with linear and quadratic fits respectively. Correlations are down to 0.98 for the linear fit but a quadratic fit is seen to improve the correlation coefficient dramatically giving a correlation coefficient of 0.999.

FIGS. 17A and 17B show the results of the multivariate (PLS) calibration in the about 1190 cm^{-1} to about 980 cm^{-1} (8.4-10.2 μm) range for LRS spiked with glucose. This region selection is broad and a multivariate method is able to correlate the glucose concentration to the spectral information in the presence of interfering spectra. FIG. 17A shows a high correlation coefficient of 0.9999 for the calibration. FIG. 17B shows the results of leave-1-out cross validation, the correlation coefficient being 0.9863. These results are close to those obtained for the IMLR with a quadratic fit indicating that there is not much loss of information in selecting a narrower range of wavelengths.

CFC FLUID

The spectra of CFC fluid samples in the spectral range of about 1200 cm^{-1} to about 975 cm^{-1} (8.3-10.3 μm) is shown in FIG. 18. These spectra further emphasize the need to select wavelength bands that have absorbance bands that correlate well with glucose absorbance so that the integrated absorbance calculation is able to obtain a reasonable fit. The shifts in baseline and the absorbance bands due to the presence of other chemicals are evident.

Again, the quadratic calibration model for the CFC fluid gave the best performance. It is seen that there is considerable deterioration of the calibration fit with linear models. It is noted here that the calibration wavelength bands needed to be selected differently as compared to the case of aqueous and LRS solutions. FIG.

19A and 19B show the results of a one wavelength band and one reference wavelength band IMLR with linear and quadratic fits respectively. The darkened symbols represent CFC fluid collected from the diabetic rat and the non-darkened symbols represent CFC fluid collected prior to the rat becoming diabetic. The correlations are down to about 0.71 for the linear fit due to the presence of multiple interferences and possible complex interaction effects. A quadratic fit improves the correlation to 0.76 in FIG. 19B. FIGS. 20A and 20B show the results of a two wavelength band one reference wavelength band IMLR calibration with a linear and quadratic fit, respectively. The correlation with the linear calibration is about 0.73. An improvement in the correlation is obtained by inclusion of a third wavelength giving a correlation coefficient of 0.83 with the quadratic.

FIGS. 21A and 21B show the results of the multivariate (PLS) calibration in the about 1190 cm^{-1} to about 980 cm^{-1} (8.4-10.2 μm) range for glucose in CFC fluid. FIG. 21A shows a correlation coefficient of 0.814 for the calibration. However, FIG. 21B shows the results of leave-1-out cross validation, the correlation coefficient being down to 0.74 indicating the stability of the calibration. The pure component spectra extracted from the above measurements show excellent agreement with the absorption peaks in the pure glucose spectrum further validating the correlation with the glucose concentration. Finally, it is noted that the correlation coefficient for the multivariate analysis is close to that obtained for the quadratic fit IMLR. This indicates that most of the information that correlates with the glucose concentration is captured by the quadratic fit IMLR.

HUMAN SERUM SAMPLES

The spectra of glucose spicked human serum samples in the spectral range of about 1400 cm^{-1} to about 975 cm^{-1} (7.1-10.3 μm) are shown in FIG. 22. In particular, human serum samples (#P31876) were obtained from Fisher Scientific and restored to liquid by the addition of distilled water. The resulting serum was spiked with varied but known quantities of glucose and centrifuged for mixing.

FIGS. 23A and 23B show the results of a one-wavelength band and one reference wavelength band IMLR with linear and quadratic fits, respectively. The wavelength bands chosen for this case were about 1195 cm^{-1} to about 1185 cm^{-1} (8.37-8.44 um) for the reference wavelength band and about 1090 cm^{-1} to about 1065

cm^{-1} (9.17-9.39 um) for the glucose wavelength band. The quadratic calibration shows a better fit as shown in FIG. 23B.

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only the preferred embodiments have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected.

CLAIMS:

1. A method of measuring an amount of an organic substance contained within a biological sample, said organic substance having an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of said wavelength regions substantially correspond to an absorption band of said absorption spectrum, comprising:

(a) detecting the intensity of a number of selected wavelength bands of infrared electromagnetic radiation influenced by said organic substance contained within said biological sample with a detection system, wherein (i) each of said selected wavelength bands substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is equal to n-1 or less;

(b) generating an electrical signal in response to detecting the intensity of said number of said selected wavelength bands;

(c) receiving said electrical signal with a signal processor configured to process said electrical signal with a quantification algorithm; and

(d) processing said electrical signal with said quantification algorithm so as to provide a measurement of said amount of said organic substance contained within said biological sample.

2. The method of claim 1, wherein:

(a) includes detecting the intensity of said selected wavelength bands of infrared electromagnetic radiation influenced by glucose with said detection system.

3. The method of claim 1, further comprising:

(e) collecting said biological sample from a mammal.

4. The method of claim 1, wherein:

said quantification algorithm of (c) includes dividing a first wavelength band integrated absorbance value by a reference wavelength band integrated absorbance value.

5. The method of claim 4, wherein:

said quantification algorithm of (c) further includes dividing a second wavelength band integrated absorbance value by said reference wavelength band integrated absorbance value.

6. The method of claim 1, wherein:

(a) includes detecting the intensity of (i) about 1090 cm^{-1} to about 1075 cm^{-1} wavelength band of infrared electromagnetic radiation and (ii) about a 1095 cm^{-1} to about 1090 cm^{-1} wavelength band of infrared electromagnetic radiation.

7. The method of claim 1, wherein:

(a) includes detecting the intensity of (i) about a 1090 cm^{-1} to about 1075 cm^{-1} wavelength band of infrared electromagnetic radiation, (ii) about a 1175 cm^{-1} to about 1137 cm^{-1} wavelength band of infrared electromagnetic radiation, and (iii) about a 1180 cm^{-1} to about 1170 cm^{-1} wavelength band of infrared electromagnetic radiation.

8. The method of claim 1, wherein:

said number of selected wavelength bands of (a) are within a range defined by about 1400 cm^{-1} to about 950 cm^{-1}.

9. A method of measuring an amount of glucose in a biological fluid, wherein said glucose has an infrared absorption spectrum which includes a set (n) of infrared wavelength regions, wherein each of said infrared wavelength regions substantially correspond to an infrared absorption band of said infrared absorption spectrum, comprising:

(a) detecting the transmittance of a number of selected wavelength bands of infrared electromagnetic radiation absorbed by said glucose contained within said biological fluid with a detection system, wherein (i) each of said selected wavelength bands substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is equal to n-1 or less;

(b) generating an electrical signal in response to detecting the transmittance of said infrared electromagnetic radiation;

(c) receiving said electrical signal with a signal processor configured to process said electrical signal with a quantification algorithm; and

(d) processing said electrical signal with said quantification algorithm so as to provide a measurement of said amount of said glucose contained within said biological fluid.

10. The method of claim 9, further comprising:

(e) collecting said biological fluid with a filtrate collector in fluid communication with a body fluid of a mammal.

11. The method of claim 10, wherein:

said mammal is a human.

12. The method of claim 9, wherein:

said quantification algorithm of (c) includes dividing a first wavelength band integrated absorbance value by a reference wavelength band integrated absorbance value.

13. The method of claim 9, wherein:

said quantification algorithm of (c) further includes dividing a second wavelength band integrated absorbance value by said reference wavelength band integrated absorbance value.

14. The method of claim 12, wherein:

(a) includes detecting the transmittance of (i) about a 1090 cm^{-1} to about 1075 cm^{-1} wavelength band of infrared electromagnetic radiation and (ii) about a 1095 cm^{-1} to about 1090 cm^{-1} wavelength band of infrared electromagnetic radiation.

15. The method of claim 13, wherein:

(a) includes detecting the transmittance of (i) about a 1090 cm^{-1} to about 1075 cm^{-1} wavelength band of infrared electromagnetic radiation, (ii) about a 1175 cm^{-1} to about 1137 cm^{-1} wavelength band of infrared electromagnetic radiation, and (iii) about a 1180 cm^{-1} to about 1170 cm^{-1} wavelength band of infrared electromagnetic radiation.

16. The method of claim 9, wherein:

said number of selected infrared wavelength bands of (a) are within a range defined by about 1400 cm^{-1} to about 950 cm^{-1}.

17. A method of measuring a concentration of an organic substance contained within a biological fluid, said organic substance having an infrared absorption spectrum which includes a set (n) of infrared wavelength regions, wherein each of said infrared wavelength regions substantially correspond to an infrared absorption band of said infrared absorption spectrum, comprising:

(a) detecting the transmittance of a number of selected wavelength bands of infrared electromagnetic radiation absorbed by said organic substance contained within said biological fluid with a detection system, wherein (i) each of said selected wavelength bands substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is equal to n-1 or less;

(b) generating an electrical signal in response to detecting the transmittance of said selected infrared electromagnetic radiation wavelength bands;

(c) receiving said electrical signal with a signal processor configured to process said electrical signal with a mathematical model; and

(d) processing said electrical signal with said mathematical model so as to provide a measurement of the concentration of said organic substance contained within said biological fluid.

18. The method of claim 17, wherein:

(a) includes detecting the transmittance of said selected electromagnetic radiation wavelength bands absorbed by glucose contained within said biological fluid with said detection system.

19. The method of claim 18, wherein:

said mathematical model includes the mathematical equation

$$C_g = P_0 + P_1 IAR_{\lambda,1} + P_2 IAR^2_{\lambda,1}$$

wherein (i) C_g is the mean-centered concentration of glucose in said biological fluid, (ii) P_i is a calibration constant, and (iii) $IAR_{\lambda,1}$ is a mean-centered integrated absorbance ratio of two of said selected wavelength bands.

20. The method of claim 18, wherein:

said mathematical model includes the mathematical equation

$$C_g = P_0 + P_1 IA_{\lambda,1} + P_2 IA_{\lambda,2} + P_3 IA^2_{\lambda,1} + P_4 IA^2_{\lambda,2} + P_5 IA_{\lambda,1} IA_{\lambda,2}$$

wherein (i) C_g is the mean centered concentration of glucose in said biological fluid, (ii) P_i are calibration constants, and (iii) $IA_{\lambda,1}$ and $IA_{\lambda,2}$ are the mean centered integrated absorbance for the selected wavelength band and the selected reference wavelength band.

21. The method of claim 18, wherein:

said mathematical model includes the mathematical equation

$$C_g = P_0 + P_1 IAR_{\lambda,1} + P_2 IAR_{\lambda,2} + P_3 IAR^2_{\lambda,1} + P_4 IAR^2_{\lambda,2} + P_5 IAR_{\lambda,1} IAR_{\lambda,2}$$

wherein (i) C_g is the mean-centered concentration of glucose in said biological fluid, (ii) P_i are calibration constants, and (iii) $IAR_{\lambda,j}$ is a mean-centered integrated absorbance ratio of two of said selected wavelength bands.

22. The method of claim 18, wherein:

said mathematical model includes the mathematical equation

$$C_g = P_0 + P_1IA_{\lambda,1} + P_2IA_{\lambda,2} + P_3IA_{\lambda,3} + P_4IA^2_{\lambda,1} + P_5IA^2_{\lambda,2} + P_6IA^2_{\lambda,3} + P_7IA_{\lambda,1}IA_{\lambda,2} + P_8IA_{\lambda,1}IA_{\lambda,3} + P_9IA_{\lambda,2}IA_{\lambda,3}$$

wherein (i) C_g is the mean centered concentration of glucose in said biological fluid, (ii) P_i are calibration constants, and (iii) $IA_{\lambda,j}$ is the mean centered integrated absorbance for band j.

23. A method of measuring an amount of an organic substance contained within a biological sample, said organic substance having an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of said wavelength regions substantially correspond to an absorption band of said absorption spectrum, comprising:

(a) illuminating said biological sample with infrared electromagnetic radiation, wherein said infrared electromagnetic radiation includes (i) one or more wavelength bands of said infrared electromagnetic radiation which are absorbed by said organic substance contained within said biological sample (ii) one or more reference wavelength bands which are not substantially absorbed by said organic substance contained within said biological sample;

(b) selecting a number said wavelength bands of said infrared electromagnetic radiation, wherein (i) each of said selected wavelength bands substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is a subset of (n);

(c) selecting a number of reference wavelength bands;

(d) detecting the intensity of only (i) said subset of said selected wavelength bands absorbed by said organic substance contained within said biological sample with a detection system and (ii) said number of reference wavelength bands;

(e) generating one or more electrical signals in response to detecting the intensity of only (i) said subset of said selected wavelength bands (ii) said number of reference wavelength bands;

(f) receiving said one or more electrical signals with a signal processor configured to process said electrical signals with a quantification algorithm; and

(g) processing said one or more electrical signals with said quantification algorithm so as to provide a measurement of said amount of said organic substance contained within said biological sample.

24. A method of measuring an amount of an organic substance contained within a biological sample, said organic substance having an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of said wavelength regions substantially correspond to an absorption band of said absorption spectrum, comprising:

(a) illuminating said biological sample with infrared electromagnetic radiation;

(b) detecting the intensity of said infrared electromagnetic radiation that is absorbed by said organic substance contained within said biological sample, wherein (i) said intensity detection is restricted to a number of selected wavelength bands of infrared electromagnetic radiation, (ii) each of said selected wavelength bands substantially corresponds to one of said wavelength regions, and (iii) said number of said selected wavelength bands is a subset of (n);

(c) generating an electrical signal in response to detecting the intensity of said subset of said selected wavelength bands;

(d) receiving said electrical signal with a signal processor configured to process said electrical signal with a quantification algorithm; and

(e) processing said electrical signal with said quantification algorithm so as to provide a measurement of said amount of said organic substance contained within said biological sample.

25. The method of claim 22, further comprising:

(f) detecting the intensity of one or more reference wavelengths bands of said infrared electromagnetic radiation which are not absorbed by said organic substance contained within said biological sample,

wherein (c) includes generating said electrical signal in response to detecting the intensity of said one or more reference wavelength bands.

26. A method of measuring an amount of an organic substance contained within a sample, said organic substance having an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of said wavelength regions substantially correspond to an absorption band of said absorption spectrum, comprising:

(a) illuminating said sample with infrared electromagnetic radiation, wherein said infrared electromagnetic radiation includes (i) one or more wavelength bands of said infrared electromagnetic radiation which are absorbed by said organic substance contained within said sample (ii) one or more reference wavelength bands which are substantially not absorbed by said organic substance contained within said sample;

(b) selecting a number said wavelength bands of said infrared electromagnetic radiation, wherein (i) each of said selected wavelength bands substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is a subset of (n);

(c) selecting a number of reference wavelength bands; and

(d) detecting with a detection system the intensity of only (i) said subset of said selected wavelength bands absorbed by said organic substance contained within said sample and (ii) said number of reference wavelength bands.

27. A method of measuring an amount of an organic substance contained within a biological sample, said organic substance having an infrared absorption spectrum which includes a set (n) of wavelength regions, wherein each of said wavelength regions substantially correspond to an absorption band of said absorption spectrum, comprising:

(a) illuminating said biological sample with infrared electromagnetic radiation, wherein said infrared electromagnetic radiation includes (i) one or more wavelength bands of said infrared electromagnetic radiation which are absorbed by said organic substance contained within said biological sample and (ii) one or more reference wavelength bands which are substantially not absorbed by said organic substance contained within said biological sample;

(b) selecting a number said wavelength bands of said infrared electromagnetic radiation, wherein (i) each of said selected wavelength bands

substantially corresponds to one of said wavelength regions and (ii) said number of said selected wavelength bands is a subset of (n);

(c) selecting a number of reference wavelength bands;

(d) detecting with a detection system the intensity of said infrared electromagnetic radiation; and

(e) processing with a mathematical model spectral data only from (i) said subset of said selected wavelength bands absorbed by said organic substance contained within said biological sample and (ii) said number of reference wavelength bands.

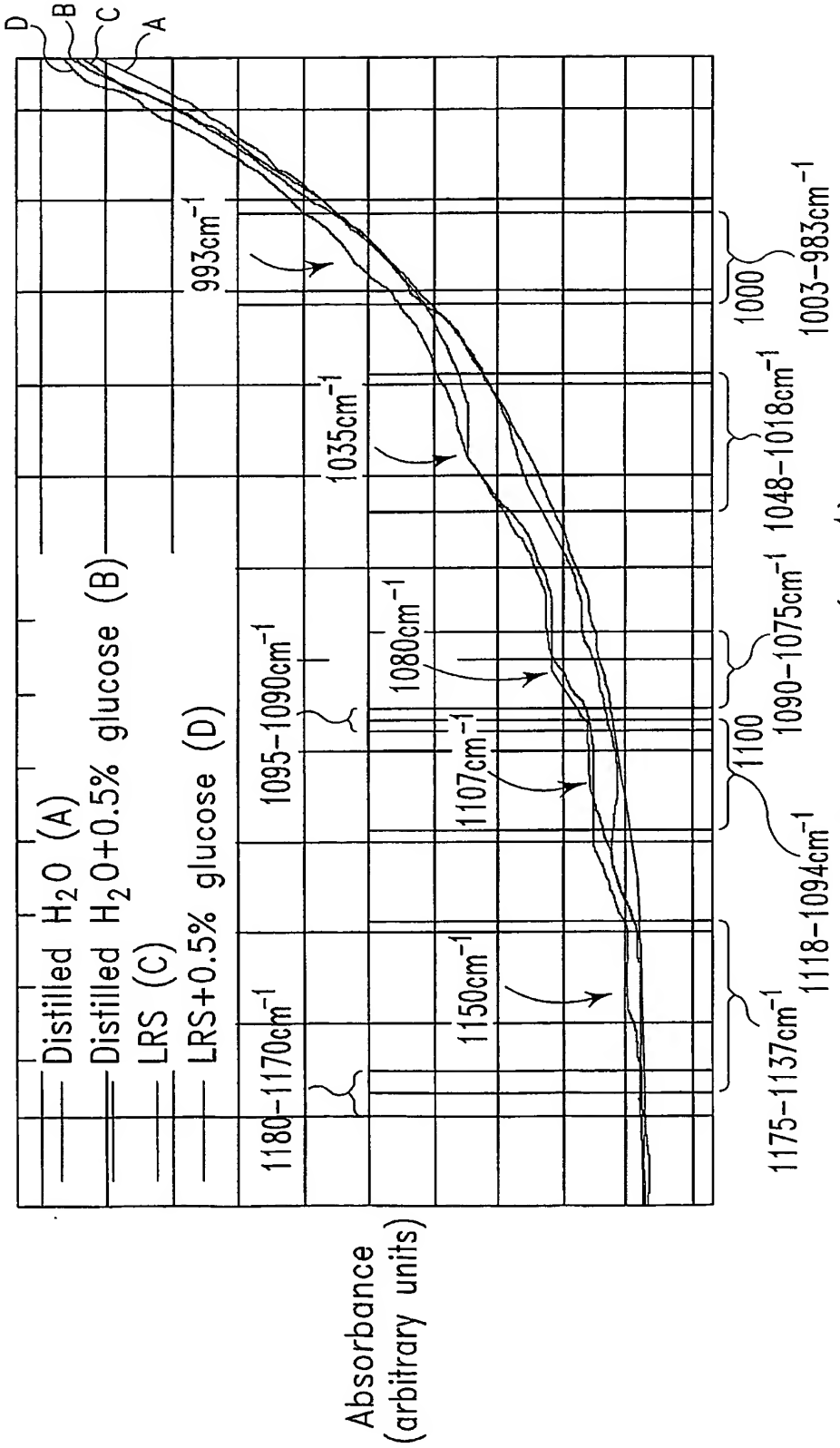
D
B
C
A
Distilled H_2O (A)
Distilled H_2O+0.5% glucose (B)
LRS (C)
LRS+0.5% glucose (D)
993cm^{-1}
1035cm^{-1}
1080cm^{-1}
1107cm^{-1}
1150cm^{-1}
1180–1170cm^{-1}
1095–1090cm^{-1}
1100
1090–1075cm^{-1}
1048–1018cm^{-1}
1000
1003–983cm^{-1}
1175–1137cm^{-1}
1118–1094cm^{-1}
Absorbance (arbitrary units)
Wavenumber (cm^{-1})

Fig. 1

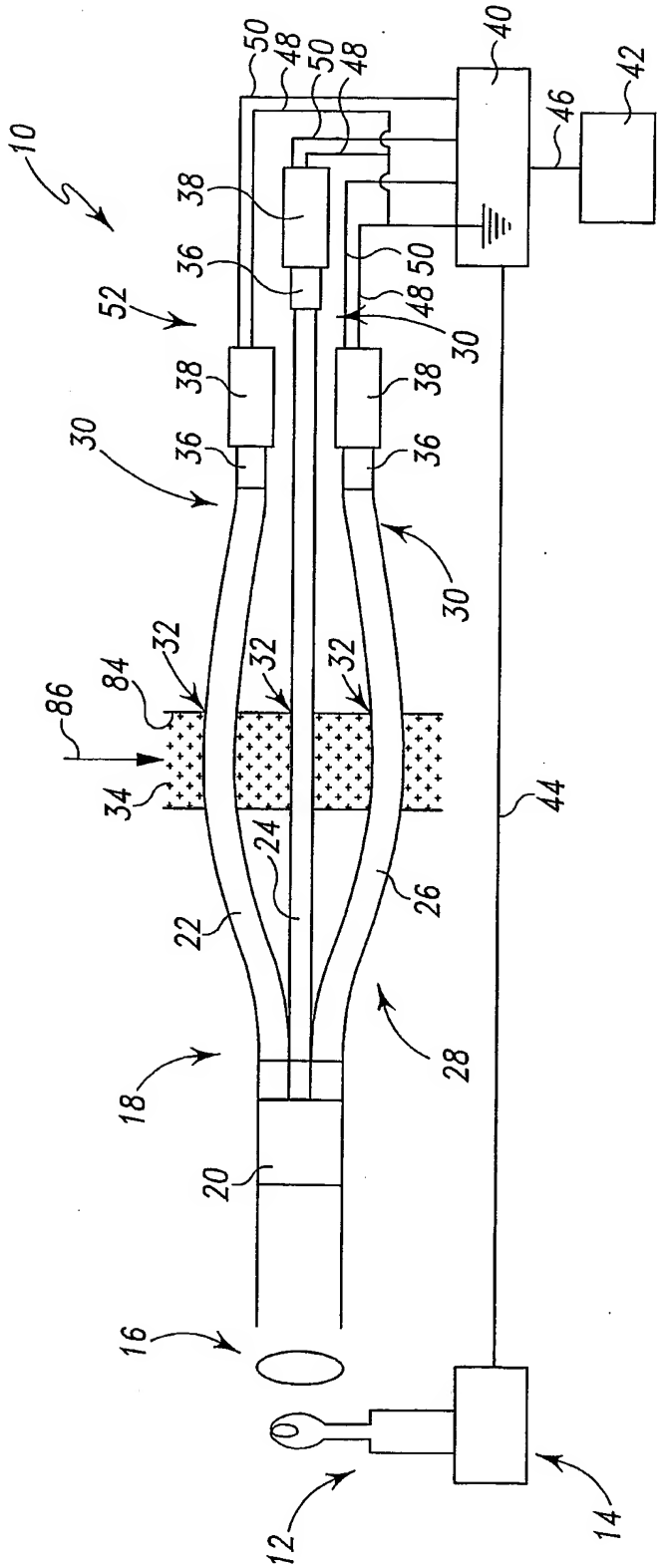

Fig. 2

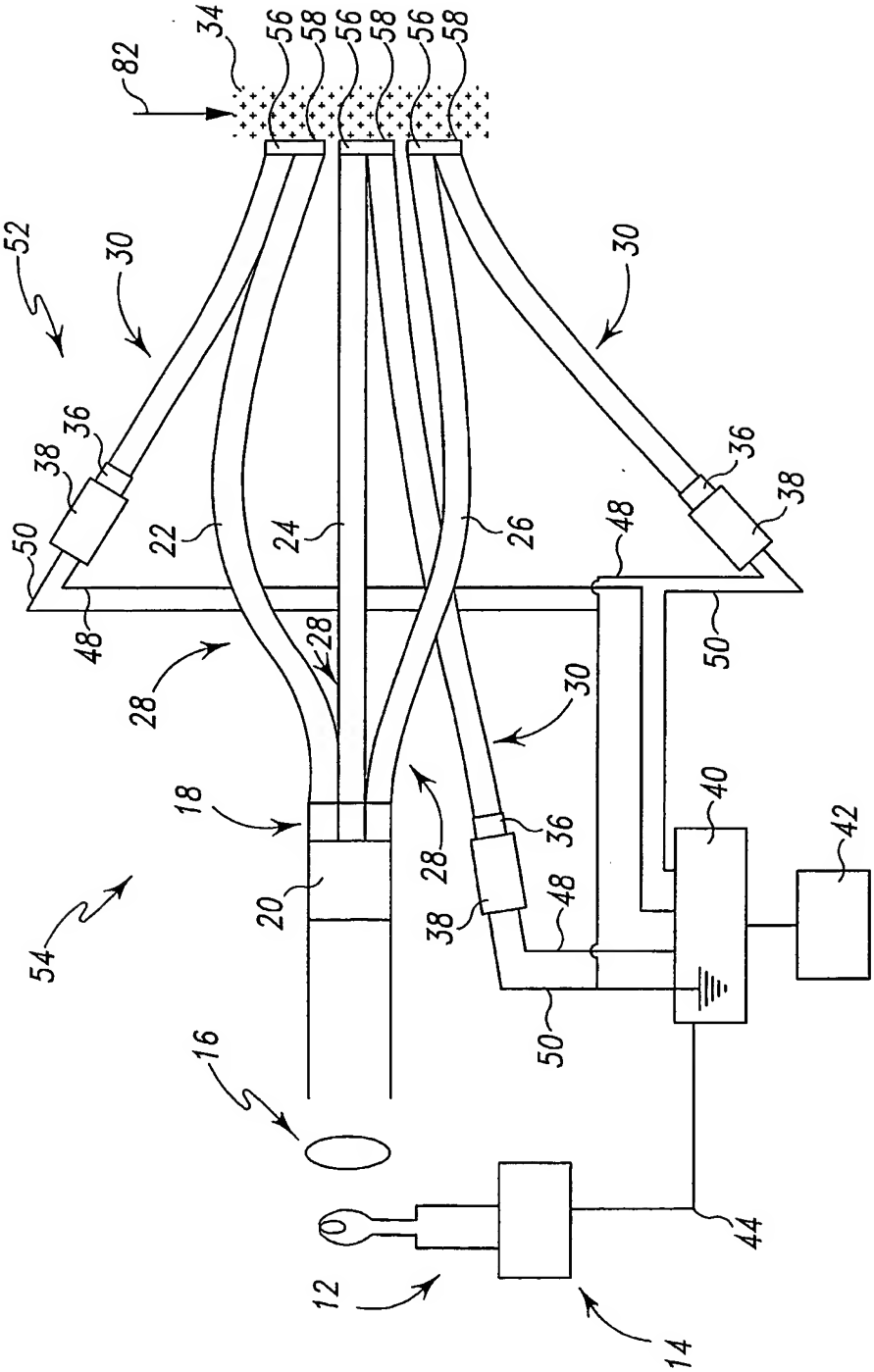
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Fig. 3

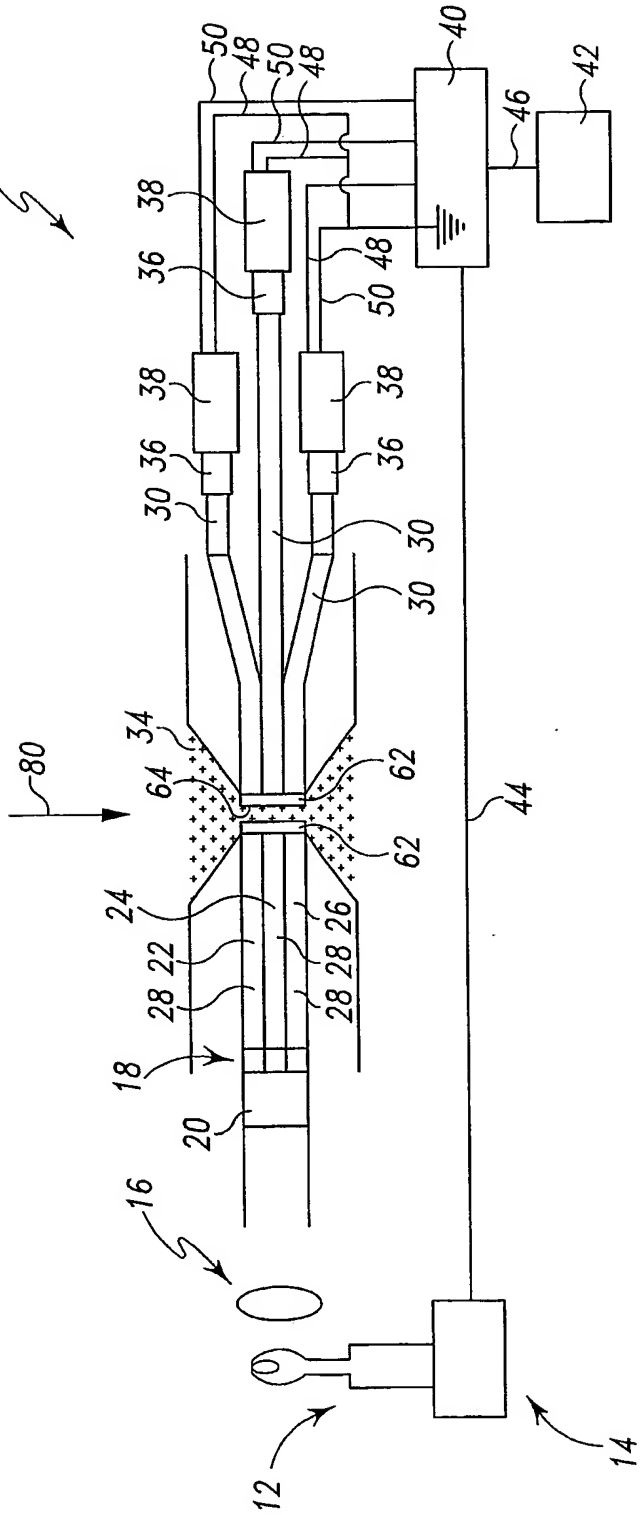
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Fig. 4

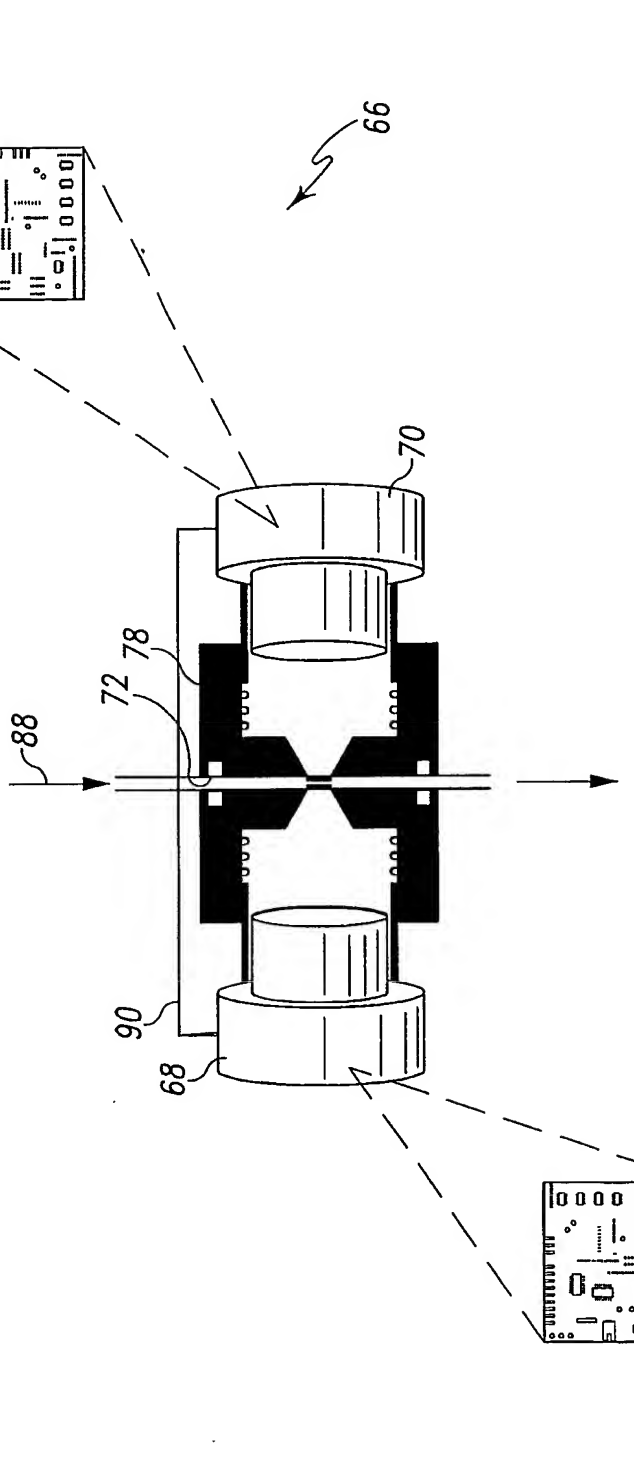

Fig. 5A

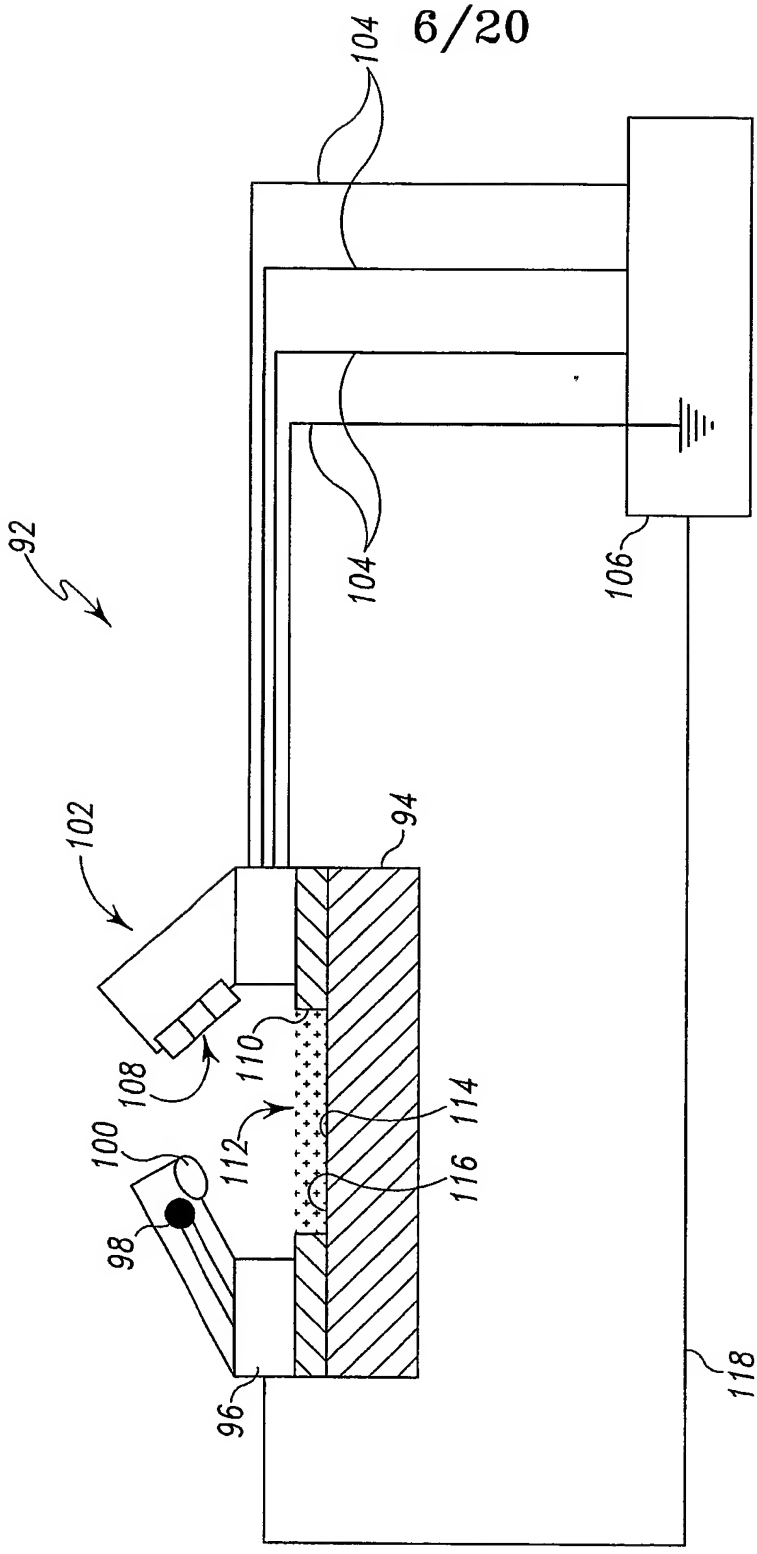

Fig. 5B

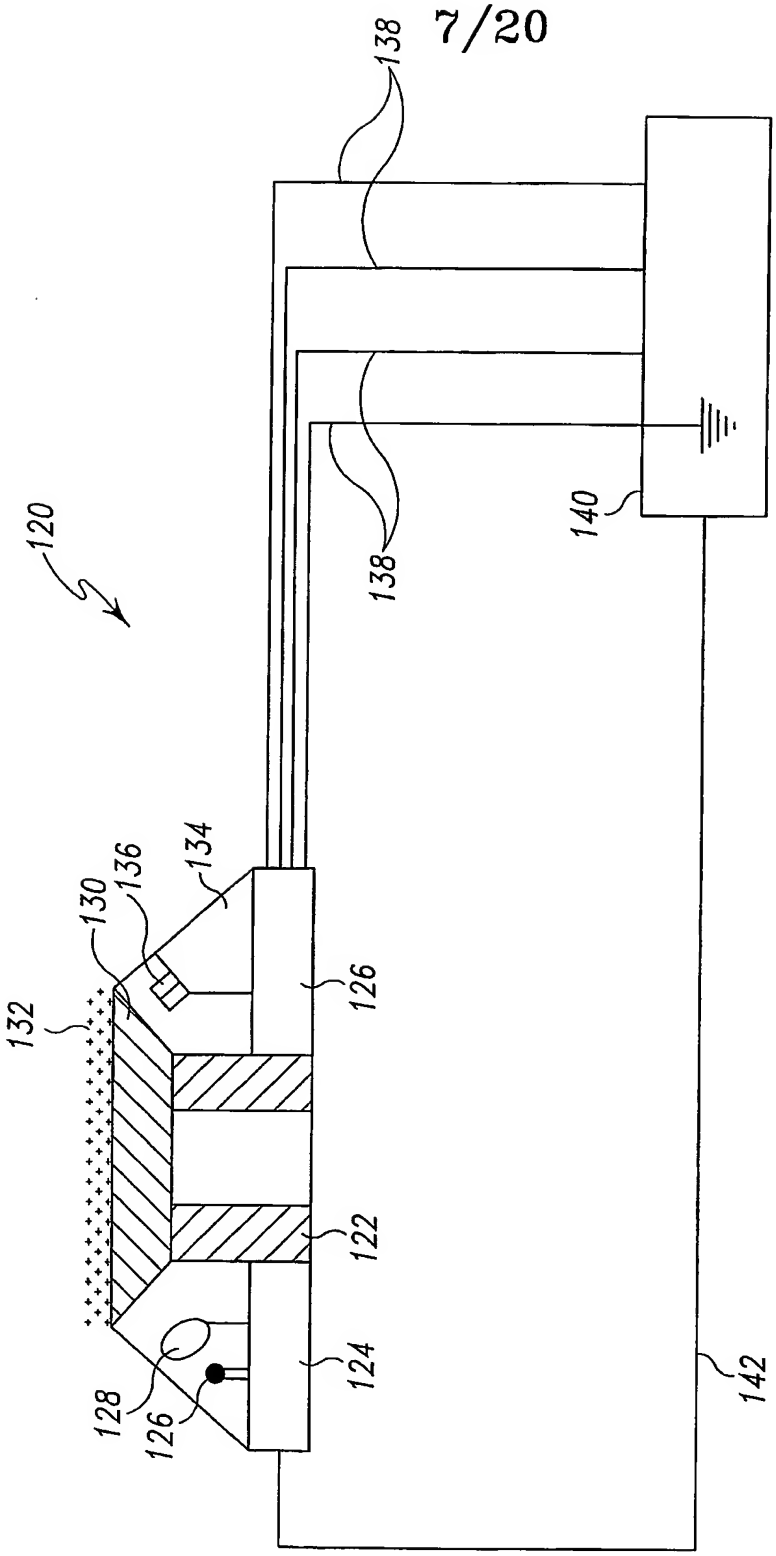

Fig. 5C

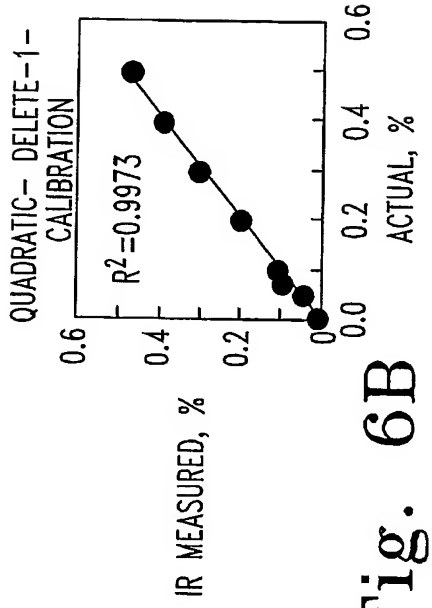

Fig. 6B

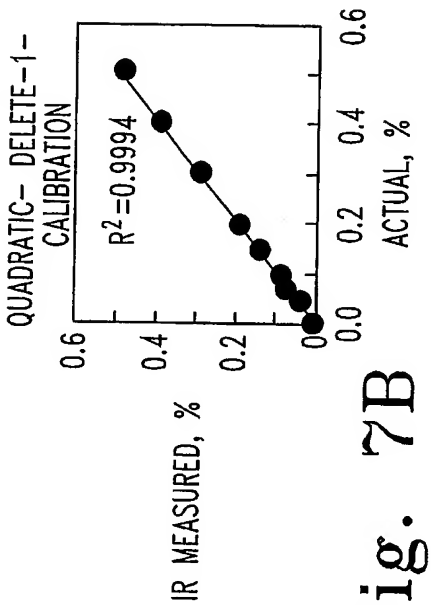

Fig. 7B

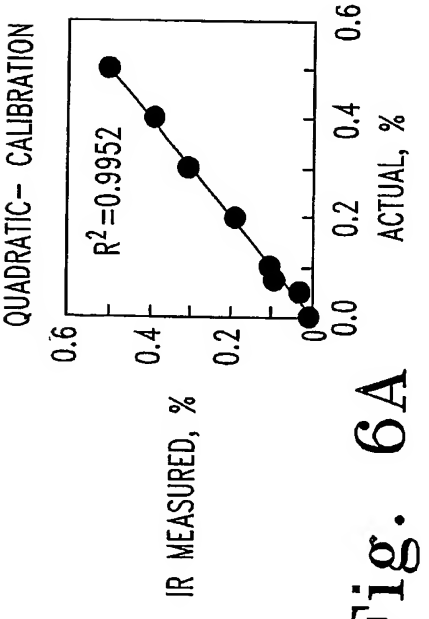

Fig. 6A

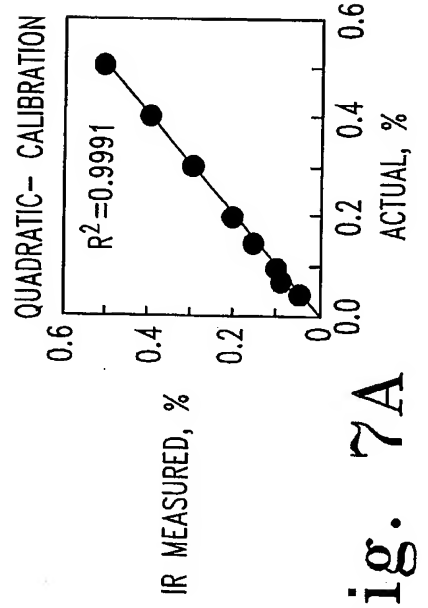

Fig. 7A

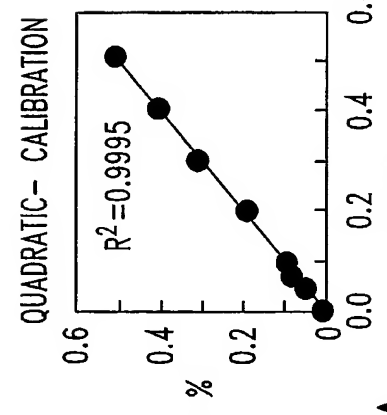

Fig. 8A

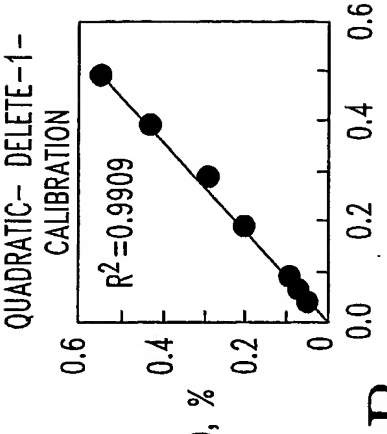

Fig. 8B

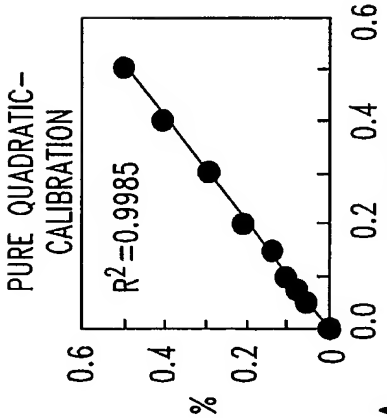

Fig. 9A

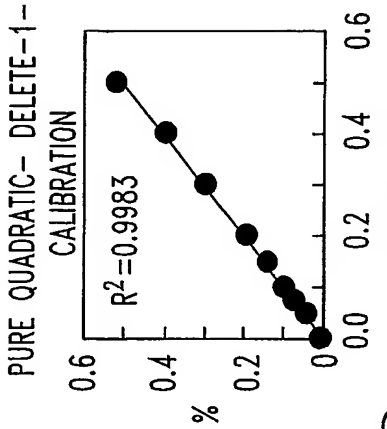

Fig. 9B

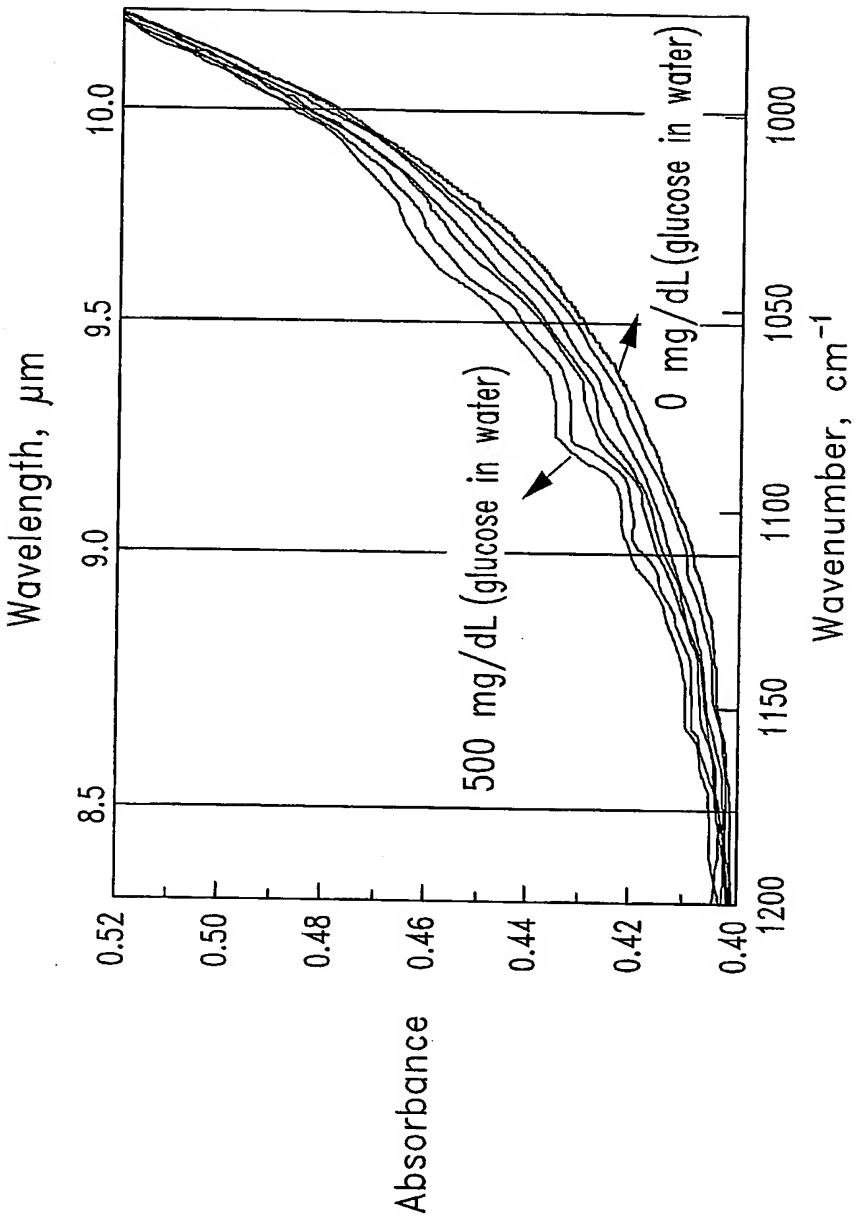
Wavelength, μm
10.0
9.5
9.0
8.5
Wavenumber, cm^{-1}
1000
1050
1100
1150
1200
Absorbance
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0 mg/dL (glucose in water)
500 mg/dL (glucose in water)

Fig. 10

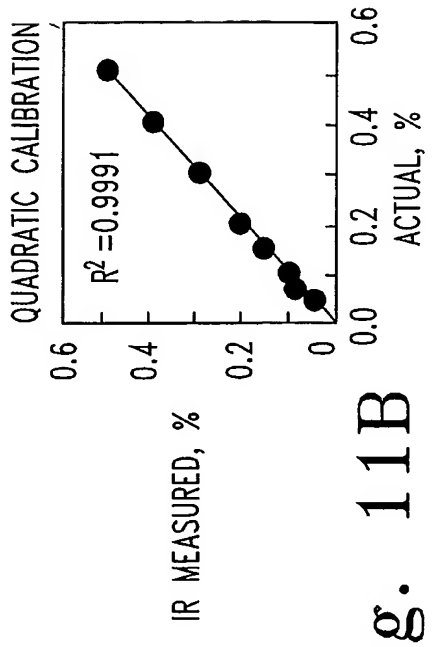

Fig. 11B

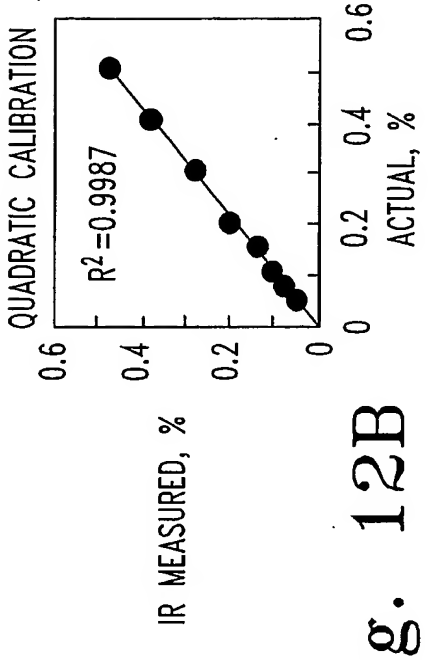

Fig. 12B

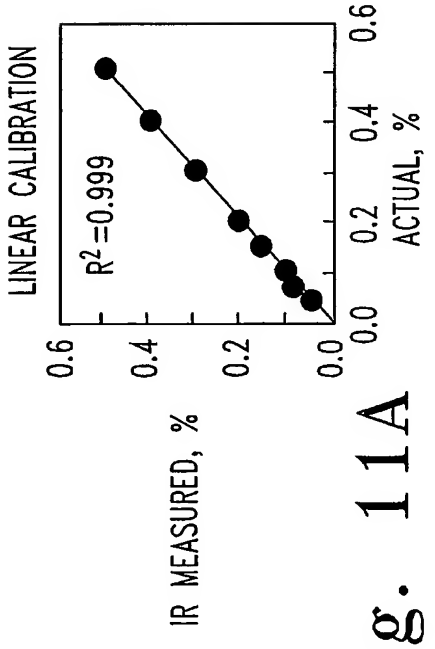

Fig. 11A

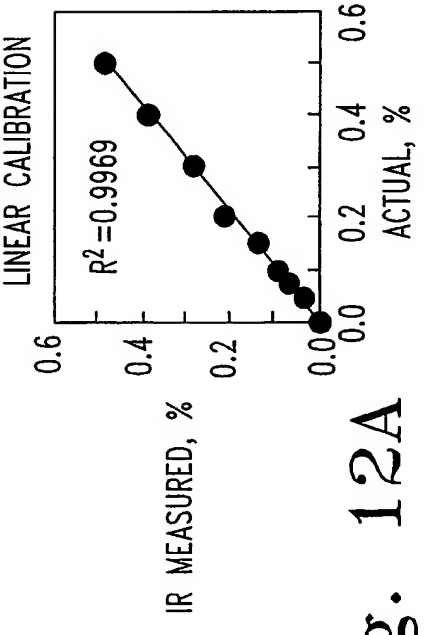

Fig. 12A

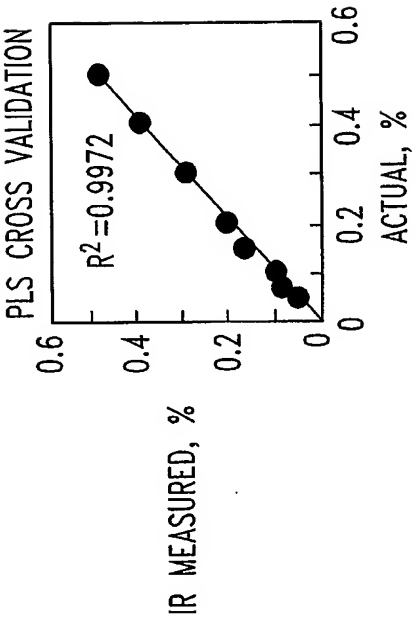

Fig. 13B

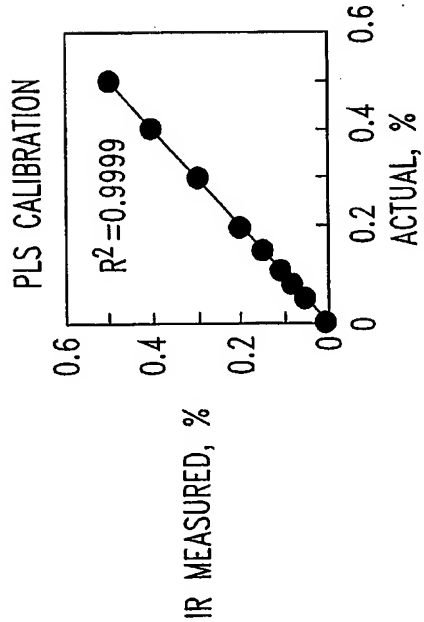

Fig. 13A

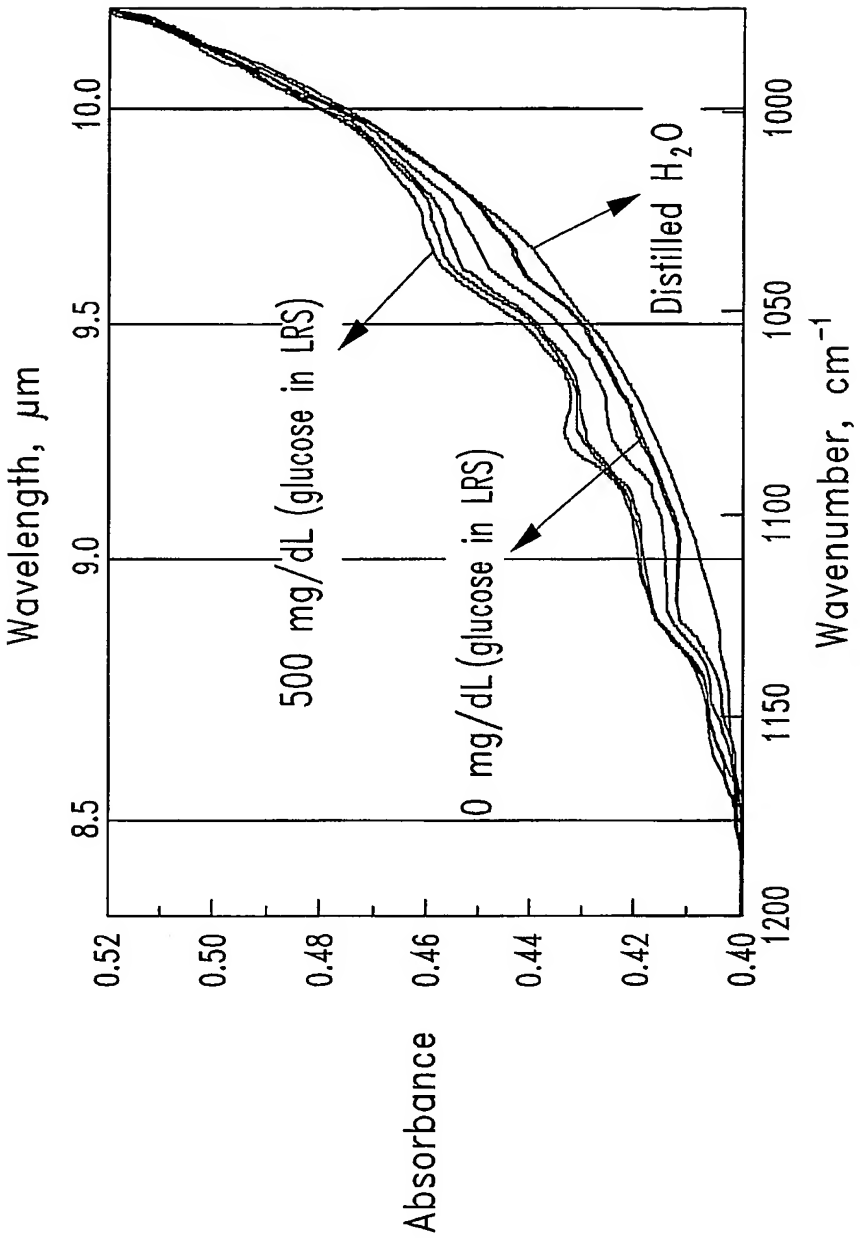
Wavelength, μm
10.0
9.5
9.0
8.5
Wavenumber, cm^{-1}
1000
1050
1100
1150
1200
Absorbance
0.52
0.50
0.48
0.46
0.44
0.42
0.40
Distilled H_2O
500 mg/dL (glucose in LRS)
0 mg/dL (glucose in LRS)

Fig. 14

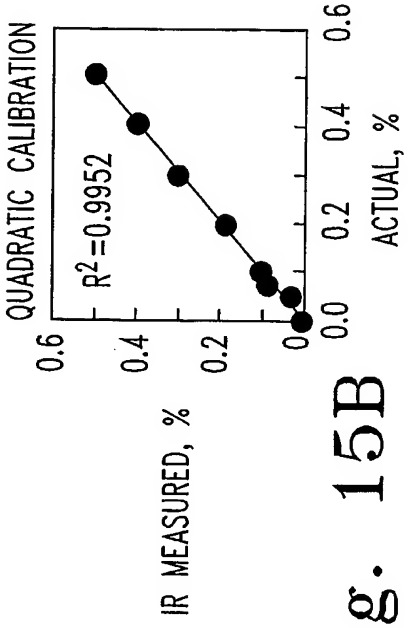

Fig. 15B

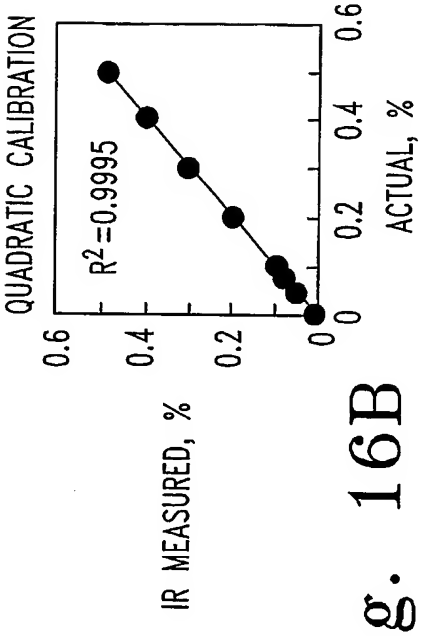

Fig. 16B

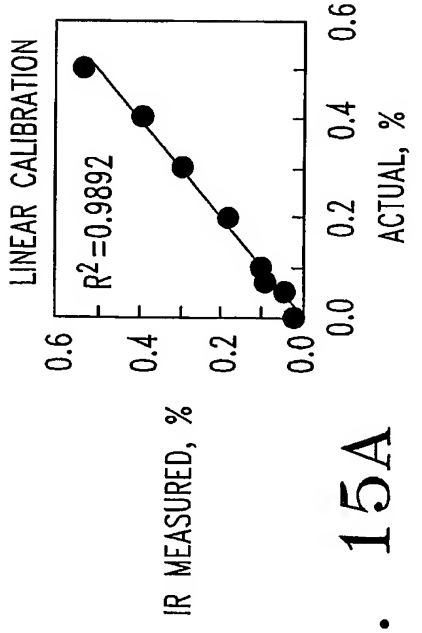

Fig. 15A

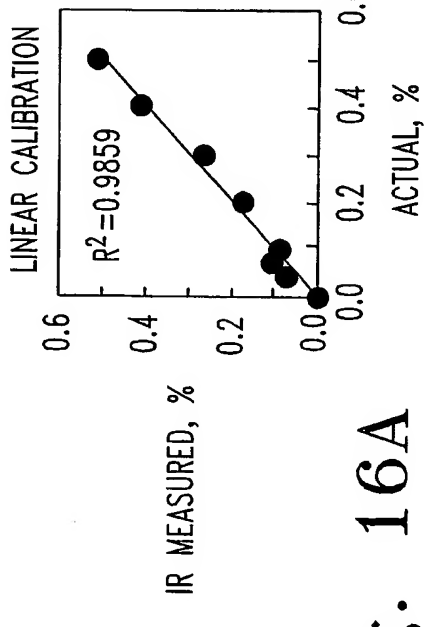

Fig. 16A

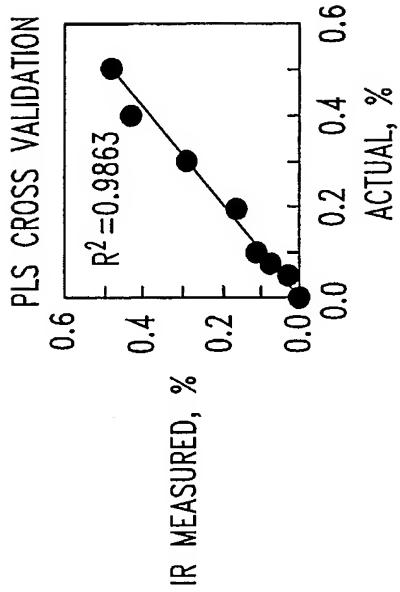

Fig. 17B

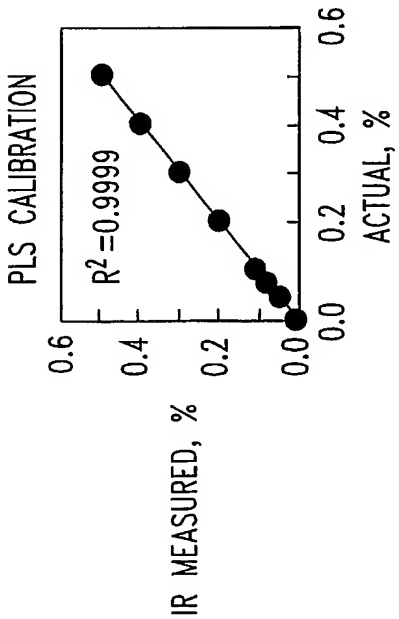

Fig. 17A

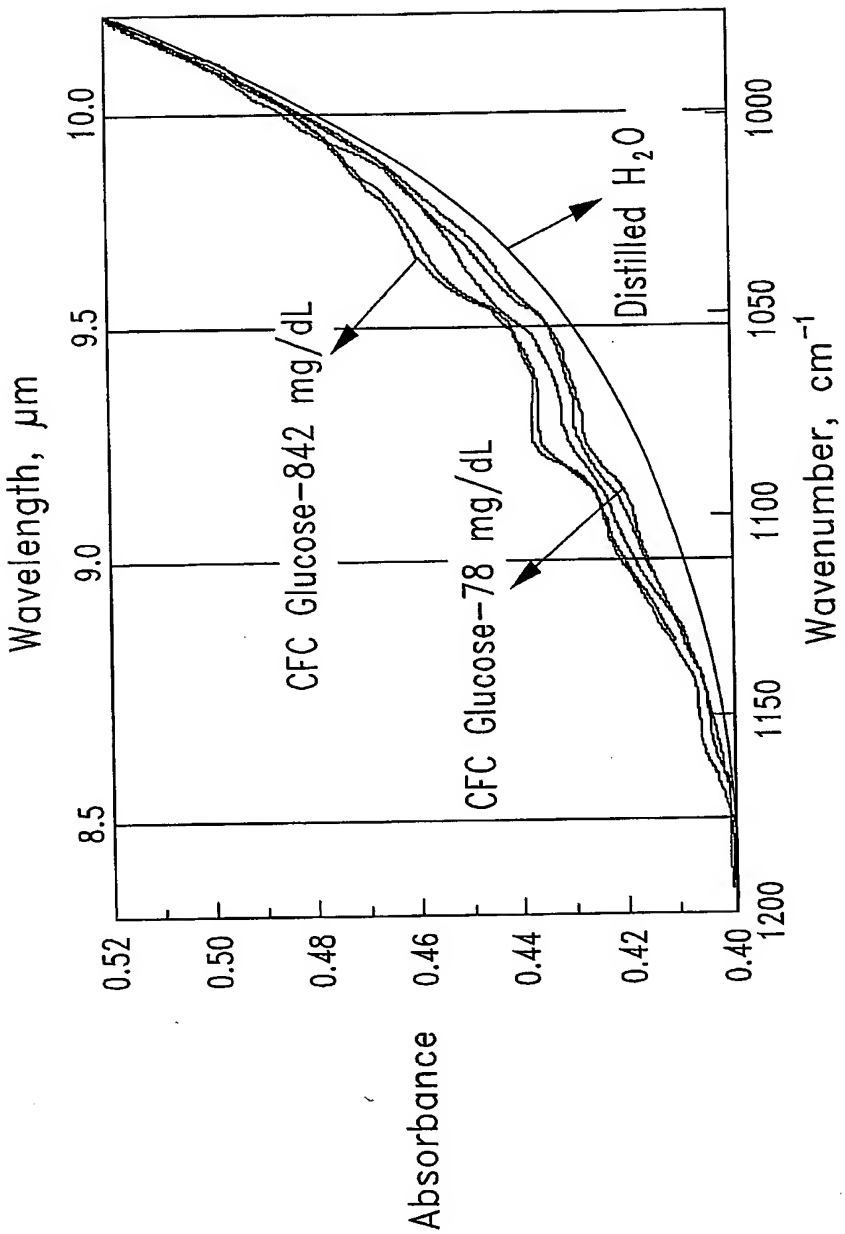
Wavelength, μm
10.0
9.5
9.0
8.5
Wavenumber, cm^{-1}
1000
1050
1100
1150
1200
Absorbance
0.52
0.50
0.48
0.46
0.44
0.42
0.40
Distilled H_2O
CFC Glucose-842 mg/dL
CFC Glucose-78 mg/dL

Fig. 18

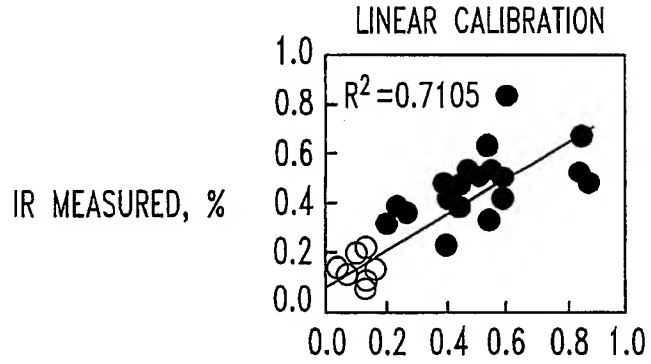

Fig. 19A

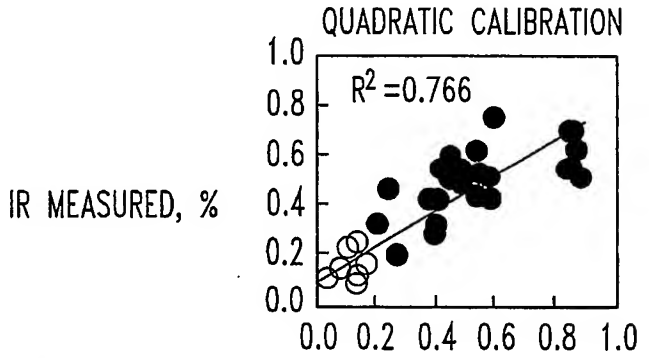

Fig. 19B

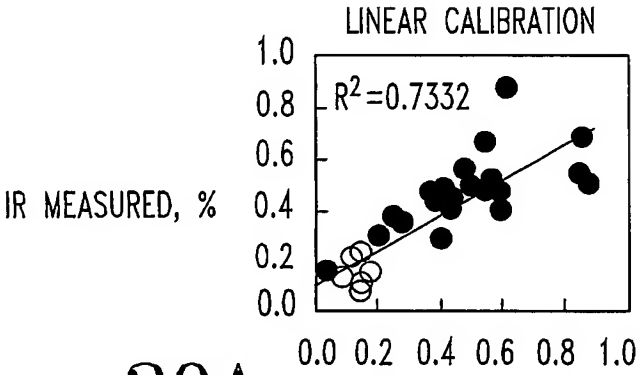

Fig. 20A

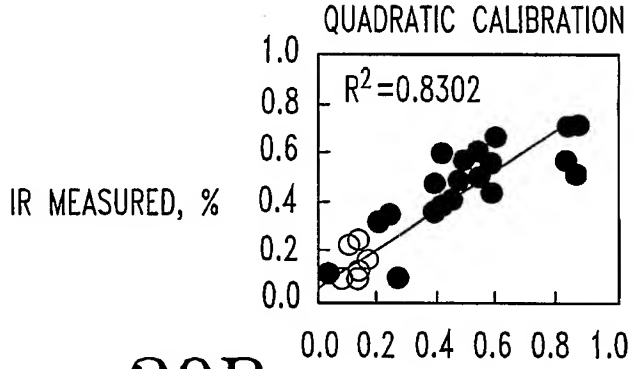

Fig. 20B

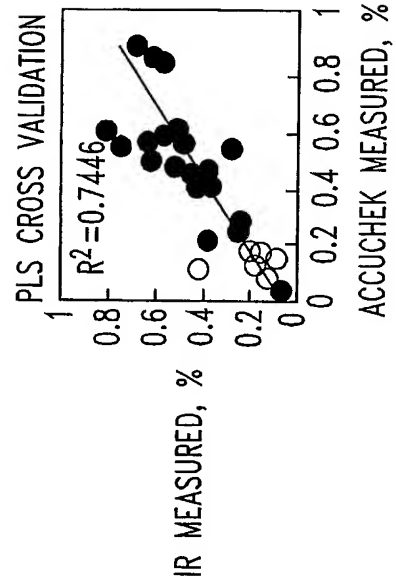

Fig. 21B

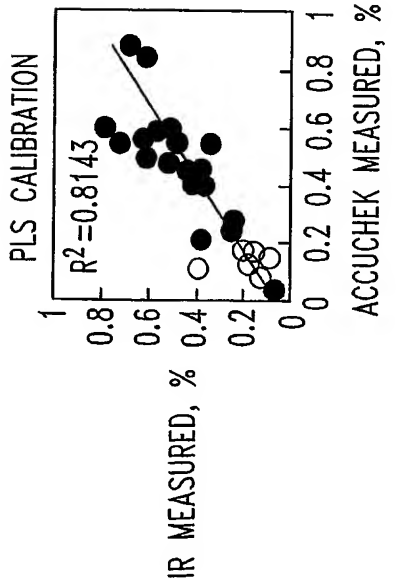

Fig. 21A

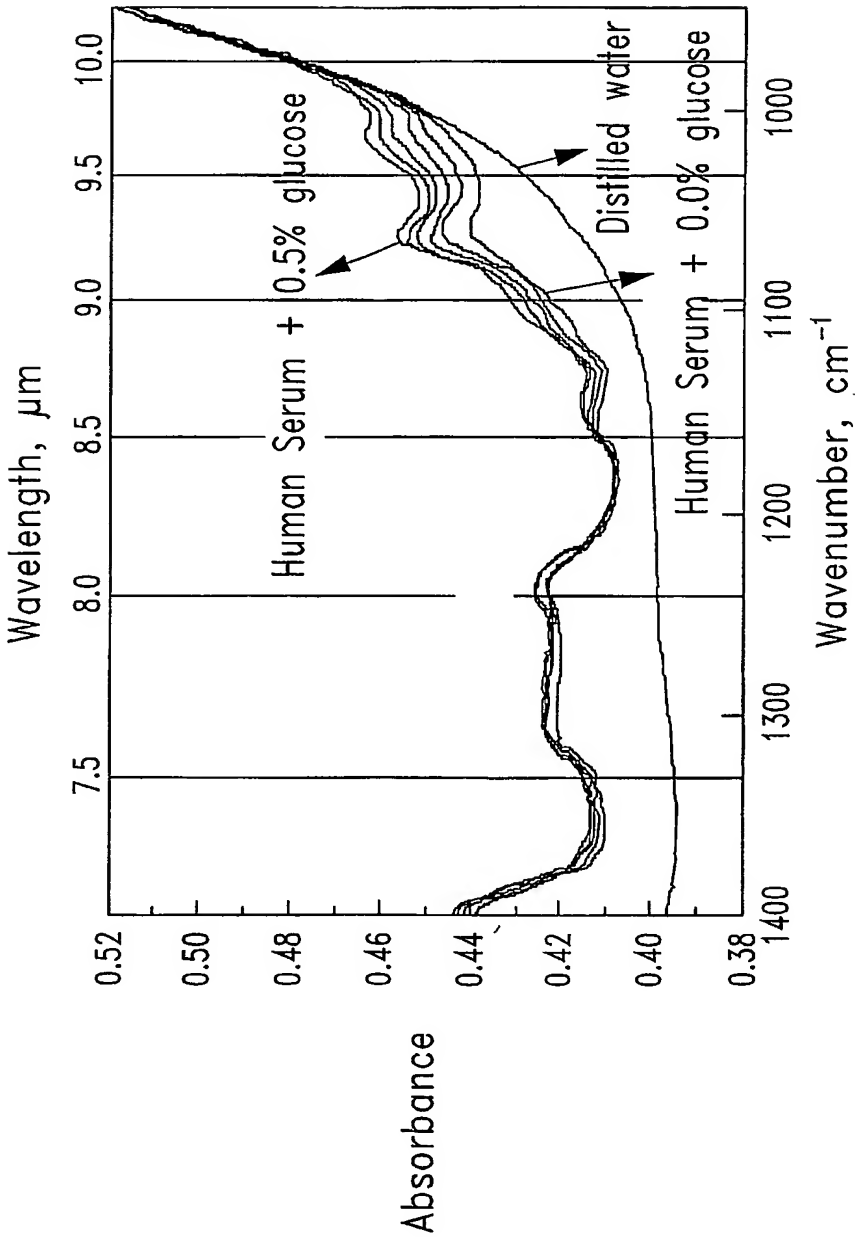
Wavelength, μm
7.5
8.0
8.5
9.0
9.5
10.0
Wavenumber, cm-1
1300
1200
1100
1000
Absorbance
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
Human Serum + 0.5% glucose
Distilled water
Human Serum + 0.0% glucose

Fig. 22

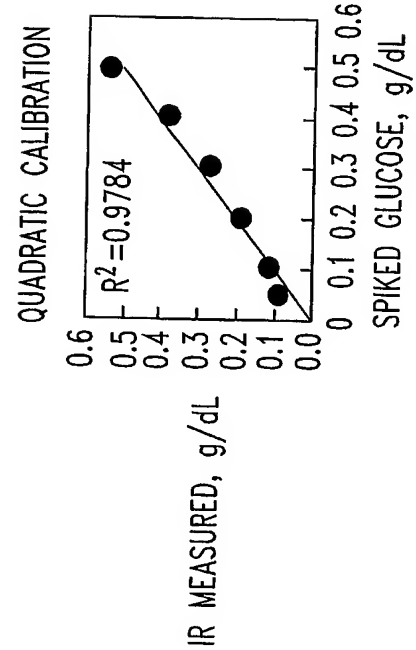

Fig. 23B

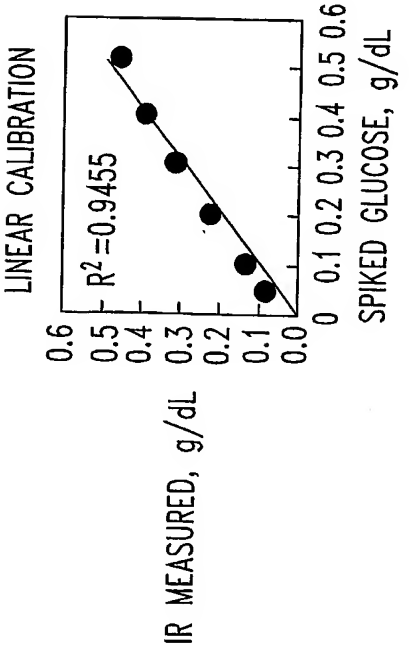

Fig. 23A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/22899

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/59

US CL :436/95, 164, 171; 356/39, 432, 436; 600/316; 250/340, 341.8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/95, 164, 171; 356/39, 432, 436; 600/316; 250/340, 341.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,743,262 A (LEPPER, JR. et al) 28 April 1998, entire document.	1-27
A	US 5,533,509 A (KOASHI et al) 09 July 1996, entire document.	1-27
A	US 5,321,265 A (BLOCK) 14 June 1994, entire document.	1-27
A	US 6,025,597 A (STERLING et al) 15 February 2000, entire document.	1-27
A	US 6,049,727 A (CROTHALL) 11 April 2000, entire document.	1-27
A	US 6,157,041 A (THOMAS et al) 05 December 2000, entire document.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
18 SEPTEMBER 2002

Date of mailing of the international search report
01 OCT 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JEFFREY R. SNAY
Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/22899

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.